ENZYMATIC CONCEPT OF ANAPHYLAXIS AND ALLERGY

and

the Role of Eosinophils in Anaphylactic Reactions related to Hormonal Alterations

BY

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For purely technical reasons the whole essay is divided into three main chapters, followed by " General Conclusions " In each chapter stress has been laid on one particular object, to make the presentation of this object more comprehensive *certain facts and theories are repeated in each chapter*

The terminology used for the designation of the intracellular proteolytic enzymes is based in principle on that accepted by M. Bergmann (1942), with some modifications For example, pepsinase, pepsin-like proteinase, Cathepsin I, and protease are synonyms, and they denominate an enzyme whose proteolytic activity is similar to but not identical with gastric pepsin. Trypsinase, trypsin-like proteinase, and Cathepsin II are synonyms, and they denominate an enzyme whose proteolytic activity is similar to but not identical with that of intestinal trypsin. The intracellular proteinases, however, differ in many respects from the corresponding enzymes of alimentary canal.

I wish to take this opportunity of expressing my thanks to the Carnegie Trust for the Universities of Scotland for meeting the expenses of production and printing of the colour photomicrograph plates as well as covering part of the cost of the printing of this book. My thanks are also due to Professor A. M. Drennan for his help and criticism and for putting at my disposal all the research facilities of his Department, to Drs F. A Richards, J S. Robson, M. MacDonald-Robson, and A. McLean for their assistance with my English and reading proofs (they have not, however, corrected the whole work); to Mr T. C Dodds for taking colour photomicrographs, and to Mr W. Robb for his supervision of the animals and for his assistance in animal experiments.

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CHAPTER I

ENZYMATIC CONCEPT OF ANAPHYLAXIS AND ALLERGY

INTRODUCTION

BEFORE entering upon the discussion of the problems of anaphylaxis and allergy, their terminology needs to be clearly defined Anaphylaxis and allergy, as will be shown below, are pathologically and ætiologically identical conditions. The only difference between them lies in the route and in the rate of penetration of antigens into the affected organism. Such dual terminology may cause confusion, hence, as a matter of convenience, the term *anaphylaxis* has been used for the phenomena of both experimental and clinical reactions due to sensitisation

The generally accepted hypothesis of anaphylaxis is based on the antigen-antibody reaction The exact nature, place, and mechanism are, however, a subject of unsettled dispute It will be shown below that manifestations of anaphylaxis are not directly caused by the antigen-antibody interaction, very often no anaphylactic symptoms accompany such interaction. In the suggested enzymatic concept of anaphylaxis the anaphylactic phenomena should be regarded rather as resulting from *the failure of the antigen-antibody reaction.* The nature and mode of action between antigen and antibody, as well as the mechanism of sensitisation and anaphylactic shock or its equivalents, are most simply explained by the alterations in intracellular and extracellular proteolysis The easiest way of presenting the enzymatic concept of anaphylaxis is to give a brief description of the nature and the mode of action of antigen and antibody, and of the mechanism of sensitisation and anaphylactic shock The basic principles of this concept are *not new, they were suggested by* Biedl *and* Kraus *in* 1909 and were supported by other workers during the following fifteen years. Contemporary knowledge of cellular biochemistry and structure was inadequately advanced to permit of sufficient supporting evidence, and this hypothesis has not been generally accepted

ANTIGEN

Definition and Mechanism of Action.

According to Topley and Wilson (1936) the definition of antigen is as follows An antigen is any substance which, when introduced parenterally into the animal tissues, stimulates the production of antibody, and which, when mixed with that antibody, reacts with it in some observable way. This definition lays particular stress on antibody In the enzymatic approach to anaphylaxis the stress is laid upon the close interdependence of antigen and sensitisation which is the basic feature of anaphylaxis Sensitisation is a condition in which toxic enzymes appear in the cells involved as a result of antigenic action. Antigen may therefore be described as any substance which, when introduced into the animal organism, whether orally or parenterally, is able to induce the formation of (1) a toxic enzyme system in the cells liable to be sensitised, and (2) specific antibodies in the tissues concerned. These two fundamental features of antigen are not necessarily coupled with each other, *i e*, the production of specific antibodies may take place in an organism in which sensitisation has not occurred, and vice versa. This is a brief outline of the hypothesis which is discussed in this work.

Antigen may enter the organism by several portals of entry, *e.g*, skin, lungs, alimentary canal, etc., or it may originate in the organism's own proteins In the latter case the protein is altered in some way, whether chemically or physico-chemically, and the organism thereafter treats it as a foreign protein. For example, during and after a surgical operation material from damaged cells enters the circulation and may exert its antigenic activity upon various organs

The chemical constitution of antigens varies considerably. The substance *which actually exerts antigenic activity* on the affected cells is always a protein, either wholly or in part. The substance which originally entered the organism may not *be a protein and may not possess, per se, antigenic power.* Such a substance after entering the organism interacts with the organism's own proteins. The resulting compound acquires antigenic activity (*Haptens*).

Antigen, *per se,* is not toxic: when it enters an organism

not previously sensitised it produces no anaphylactic reaction. Antigen must be distinguished from a group of substances which are not antigens but which, when injected into a non-sensitised organism, produce symptoms identical with those of anaphylaxis Examples of this pseudo-antigenic activity are the reaction following the intravenous injection of chloroform (p. 43), peptone shock (p. 30), and the reaction following intravenous injection of human gamma globulin into non-sensitised dogs or men (p. 42). The difference between antigenic and pseudo-antigenic actions is of fundamental importance, because it explains the mechanism of anaphylactic reaction The sensitising dose of antigen entering the cell for the first time causes toxic protease to appear in the cell. A second ("shock") dose of the antigen will then initiate toxic proteolysis which liberates intracellularly toxic breakdown products. By contrast, in pseudo-antigenic action, the pathological proteolysis is begun without previous appearance of toxic enzymes (for details see p. 40)

Fate of Antigen in the Body.

The antigenic protein, in its initial invasion in experimental anaphylaxis, as well as in clinical allergy, may be distributed all over the organism It was demonstrated by the radioactive tracer technique that practically all organs show the presence of radioactive antigen (Crampton *et al*, 1950; Haurowitz *et al*, 1951), and that tracer antigen is demonstrable in the cytoplasm as well as in the nucleus of the cells (Coons *et al*, 1951) Another group of experiments with radioactive antigen showed that soon after its injection into specifically sensitised rabbits radioactive antigen appears first in the microsomes and later in the mitochondria (site of numerous enzymes (Peters, 1952 *b*)) of the invaded cells (Haurowitz *et al.*, 1951). Since the latter are regarded as the place where the proteins are synthesised (Claude, 1943), the above workers have suggested that antigenic protein may be incorporated into the organism's own proteins The final fate of the antigen in the organism is not definitely known. Experiments with antigenic proteins marked with azo-dyes showed that their molecules are first phagocytosed by polymorph leucocytes, and later that these molecules appear in the phagocytes of the reticulo-endothelial system (Heidelberger *et al.*, 1933; Sabin, 1939,

and others). Dixon *et al.* (1951), using a double marking method of proteins with azo-dyes and with radioactive I^{131}, proved that dyed proteins are somewhat different from the non-dyed proteins in so far as their transportation into the interior of the organism is concerned. They also demonstrated that antigenic protein gradually disappears from various organs *pari passu* with the rise of the titre of the specific antibodies in the blood (for explanation see p 15) The longest period in which antigen in its native form was demonstrated in the organism was 120 days after its injection (McMaster *et al.*, 1951). The ultimate fate of the antigen or its derivatives (produced by union of antigen with antibody) is presumably proteolysis. According to the theory suggested in this essay, antigenic proteins may, in suitable conditions, be incorporated into the structure of the host-cell. In such a form they may remain intracellular as long as the invaded cell lives They may or may not alter the whole character of these cells. Furthermore, this physiopathological state may be transmitted to the next generation according to the Mendelian law.

Haptens.

Primarily non-antigenic substances, such as benzoic acid derivatives, may acquire antigenic character if, after entering the organism, they couple themselves with the organism's own protein molecules . these newly formed antigenic compounds are designated as *haptens* (Landsteiner, 1920) or *derivative* antigens (Chase, 1952) These attached substances are then called *determinant groups* because they confer specificity upon the proteins with which they are linked (Marrack, 1950). Haptens can also be produced artificially *in vitro*, and their specificity may be tested serologically by using specific antisera (Landsteiner *et al.*, 1927, 1929, 1932, 1934, 1939 , Pauling *et al.*, 1942 , Pauling *et al.*, 1944).

ANTIBODY

Definition and Mechanism of Action.

Antibodies are proteins of globulin type, some of which are gamma globulin (Kass, 1945 ; and others) They appear in the body as a result of antigenic action and are marked

chemically in such a way as to enable them to interact with the specific antigen, rendering it inactive (Marrack, 1950).

If the criteria for the classification of antibodies are their effects on the antigenic protein, there are at least five main types of antibody to be distinguished

1. If the new protein compound, formed from the protein of antibody and protein of antigen, disintegrates completely to the level of non-toxic catabolites, such antibody may be called *lysin*
2. If the newly formed protein molecules become insoluble in the existing chemical environment and are precipitated, such antibody is called *precipitin* (Eagle, 1935)
3. If the viscosity of the newly formed protein molecules, *localised on the surface of bacteria or cells, is such as* to cause aggregation of these bacteria or cells, this antibody is called *agglutinin*
4. If an antibody attaches itself to the antigen molecules on the surface of bacteria or cells, forming a layer which alters the surface-acting forces of the bacteria or cells, thus permitting phagocytosis by phagocytes, this antibody is called *opsonin* (Mudd *et al*, 1934)
5. *Complement fixation* and *toxin neutralisation* also take place as a result of attachment of antibody to the specific antigen (Rich, 1944)

The first type of reaction may be regarded as enzymatic, and the antibody concerned as proteolytic enzyme This action may take place either inside the cells or in the body fluids, and may be directed either (*a*) towards one particular type of antigenic protein (by formation of a " bridge " or by spatial adaptability of the interacting molecules (p 24)), or (*b*) towards any protein which contacts them in their active form. This does not mean that all proteolytic enzymes existing in the organism are proteolytic antibodies (for details see p. 8.) The tryptic action of antibodies and enzymes is the main topic in the further discussion on sensitisation and anaphylaxis. The second, third, and fourth types of antibody may be regarded as acting through physico-chemical channels; the changes in solubility and viscosity result from union of antigenic protein with the protein of antibody. The mode of action of the fifth

type of antibody is as yet not clearly understood; its action, unlike that of the first type, is usually antigen-specific.

The *task of the last four types of antibody* is to neutralise the antigen by one of the above-mentioned forms of antigen-antibody reaction. By so doing, the antigen-antibody reaction prevents the spread of *antigen and so protects the organism* from sensitisation by the initial dose or from anaphylactic reaction if the organism is already sensitised.

Site of Antibody Production.

There are various reports claiming that all cells of a normal organism are able to produce antibodies (Cannon *et al.*, 1932; Walsh *et al.*, 1934, Westwater, 1940, Oakley *et al.*, 1949). However, their evidence is not conclusive (critically reviewed by Cannon *et al.*, 1934). The main source of the production of antibodies is lymphoid tissue (Drinker *et al.*, 1941, Ehrich *et al*, 1942, Dougherty *et al*, 1944 *a*, and others). Whether the whole lymphatic system or only part of it is engaged in *the production of antibodies depends on several factors. If* the regional lymphoid tissue is sufficiently active in neutralisation of the invading antigen (by antigen-antibody reaction) so that antigen cannot pass the regional lymphatic barrier, the actual task of antibody production rests with the regional lymph glands. If, however, antigen penetrates through the lymphoid barrier or is brought into the interior of the organism by hæmatogenous spread, the whole lymphatic system may be engaged in manufacturing antibodies (Howell, 1928; McMaster *et al.*, 1935; Sabin, 1939). The lymphatic tissue invaded by antigen may hypertrophy as a result of its increased activity. Such hypertrophy presents as a local or general enlargement of the lymph nodes. Thus parenteral administration of foreign protein *in animals or men may produce enlargement of the* spleen and lymph nodes (Epstein, 1929, Wiseman, 1931; Rich *et al.*, 1939).

Local Immunity.

The interior of the organism may be invaded by the antigen if it travels via blood capillaries, or if antigen-antibody reaction fails to neutralise the penetrating antigen in the regional lymphoid barrier or in the body fluids. In both cases various organs are invaded by the antigen and theoretically all these

invaded organs may produce antibodies Even if these organs are able to manufacture specific antibodies, the amount of these antibodies contributed to the general pool in the blood can be of little significance The ability of other than lymphoid tissues to produce antibodies may explain their *local immunity* Such a mechanism of local immunity is not generally accepted (critically reviewed by Cannon *et al*, 1934) Another more plausible explanation of *local immunity* suggests a mechanism similar to that operating in *local fixation* of antibodies in inflamed tissues from the blood (Fox, 1936) The enzymatic approach to local immunity offers a different explanation. The presence of normal proteolytic enzymes in normal cells, possessing the power of rapid proteolysis of any protein, antigenic or not, to the level of non-toxic catabolites, deprives the antigenic molecules of their toxicity before they can perform their *sensitising action* Thus, *according to this theory*, *local immunity* depends upon the ability of immune organs (normal organs) to hydrolyse the antigenic protein by their non-specific proteinases; organs whose proteolytic enzymes are inefficient are not immune and are liable to be sensitised

Relation of Lymphatic Tissue to the Release of Antibodies.

A specific antigen travelling via the lymphatics guarded by the lymphoid tissue may be totally neutralised in the lymphoid barrier or in the body fluids by one of the five forms of antigen-antibody reaction If so, the whole incident of antigen penetration is closed, except that specific antibodies have appeared in the regional lymphoid tissue This lymphoid tissue in the normal process of ageing disintegrates and releases the antibodies into the circulation. Thus localised penetration of antigen is reflected in the body fluids in the form of free specific antibodies.

Some of the antigen may pass through the defence barrier of lymphatic tissue or it may by-pass it by travelling in the blood stream, in which case all organs, the lymphatic system included, will be invaded by antigen in highly dilute form. Non-lymphatic organs with deficient proteolytic systems become sensitised. If in spite of the dilution of antigen lymphoid tissue has been sufficiently stimulated to produce specific antibodies, these will be temporarily stored there Thus lymphatic tissue becomes an important source of antibodies. The injection of C^{11}-oxygenated corticosteroids causes

rapid dissolution of the lymphoid tissue (Selye, 1946); any antibodies contained therein will be released in such case into the circulation (Dougherty *et al.*, 1944 *a*, 1944 *b*; White *et al.*, 1946). If, however, the dilution of the antigen is too high or the lymphatic tissue insufficiently reactive, no specific antibodies will be formed, and the injection of lympholytic corticosteroids will not influence the titre of the specific antibodies in the blood. This may explain why the findings of the above-mentioned workers have not been confirmed by others (De Vries, 1950; Dougherty, 1951).

Specificity of Proteolytic Antibodies.

Intracellular proteolytic enzymes are present in varying quantities in all the tissues of a normal organism, whether or not antigens have been encountered. These proteolytic enzymes are non-specific in hydrolysis, *i.e.*, any protein which makes contact with them (in their active form) is split very rapidly to the level of non-toxic catabolites. They can also catabolise antigenic proteins, yet they must not be regarded as proteolytic antibodies because the hydrolysis of the antigenic protein has no specific character. These proteolytic intracellular enzymes are designated by various terms, such as *proteinase, trypsinase, Cathepsin II*, or by various other names according to their hydrolytic action on the synthetic substrates.

The specific proteolytic antibodies are those proteolytic enzymes which can proteolyse specific antigen only, which were not present in the cell before the entry of antigen, and which by the hydrolysis of antigen abolish its toxic action. The specific proteolytic antibody may react with antigen in at least one of two ways

1. Formation of a bridge between molecule of enzyme and molecule of substrate by a particular polyvalent metal ion.
2. Spatial adaptability of the "binding planes" between two interacting molecules.

Details of these two theories are more fully discussed on page 24.

Proteolytic antibodies neutralise antigen by hydrolysing its protein to the *level of non-toxic catabolites.* Specific proteolytic antibodies have to be clearly distinguished from *specific*

proteolytic toxic enzymes. Toxic proteolytic enzymes act only on specific antigens, but their final *breakdown products are toxic.* In other words, the specific proteolytic antibodies have the task of defending the organism against the noxious actions of antigen, and the specific proteolytic toxic enzymes damage the organism by their toxic end products

The distinction between specific proteolytic antibody and specific proteolytic toxic enzyme has fundamental significance in the enzymatic concept of anaphylaxis, this theory admits two factors separately operating in the pathogenesis of anaphylaxis

1. *Antigen-antibody Reaction* —This requires specific antibody, and its task is to prevent anaphylactic sensitisation in the affected cells
2. *Formation of a Proteolytic Toxic Enzyme System* —This constitutes the basic phenomenon of anaphylactic sensitisation

Both these reactions are fully discussed in the chapter on sensitisation

According to the enzymatic approach to anaphylaxis the anaphylactically sensitised cells manufacture a specific toxic protease Once the toxic protease is formed in the cell, it remains in this cell for the rest of its life, this enzymatic pattern may even be stretched beyond the life of that cell by the incorporation of this pathological enzymatic pattern into *the biochemical phenotype* (Gulick, 1944) In such a way the enzymatic constitution may be transmitted to the next generation of cells and to the next generation of the whole organism. This may explain the hereditary character of anaphylactic phenomena

SENSITISATION

Mechanisms of Sensitisation.

Sensitisation takes place in the cells of so-called *shock organs.* These are the tissues where most of the anaphylactic reaction takes place. According to the enzymatic concept of anaphylaxis there are two main mechanisms of anaphylactic sensitisation :

1. The invasion by the sensitising dose of antigen takes plac

via the lymphatics from various portals of entry, at a slow rate (*e.g.*, from respiratory tract, alimentary canal, skin, etc.).

2. The sensitising dose invades the organism via blood capillaries and reaches the interior of the organism at a rapid rate, by-passing the lymphatic barrier (*e.g.*, *by intravenous injection*).

The first type of sensitisation is regarded by various allergists as occurring in human allergy. The second type of sensitisation is thought to take place in experimental anaphylaxis. Such a classification, however, is not justified, since in human allergy, e g , in serum sickness, an "anaphylactic" mechanism operates; vice versa, in experimental anaphylaxis, the sensitising dose of antigen may be introduced into the animal organism in the *same manner as in human allergy* *Thus the main difference* between these two forms of anaphylaxis lies in the route taken by the antigen and hence the rapidity with which it reaches the shock organs

Sensitisation by the Mechanism of Slow Spread of Antigen.

In this type of sensitisation antigen travels along the lymphatics. Most or all of its molecules are arrested by antigen-antibody reaction in the lymph nodes (p 8), the cells responsible being lymphocytes, plasma cells, and the cells of the reticulo-endothelial system (Sabin, 1939 , Oakley *et al.*, 1949 , and others). This arrest results in complete, or almost complete, elimination of the penetrating antigen from the circulating lymph In these circumstances sensitisation does not take place at all In the course of a normal life the organism is continuously invaded by various antigens, but the lymphatic barrier prevents their further penetration. Where the antigen-antibody reaction *is* inadequate (p. 12), some of the antigenic molecules may escape this defence mechanism and reach the blood stream via the thoracic duct. These very minute amounts of circulating non-neutralised antigenic molecules *penetrate the interior of almost all cells* in the organism (Coons *et al.*, 1951). Those cells which originally possess sufficiently active intracellular non-specific proteinases (Cathepsin II) can break down the invading antigenic protein to non-toxic catabolites. These are normal cells, and they may

be described as *insensitive* to a given antigen or as *immunised* to it The cells which are liable to be sensitised are those which, at the time of the initial invasion of antigen, do not possess sufficiently active non-specific proteinases capable of proteolysing and thus getting rid of the antigenic protein Such cells are subjected to the toxic action of the unsplit antigenic molecules. The partially incapacitated proteinases of the sensitised cells may be still capable of hydrolysing the structural proteins of their own cytoplasm but they are incapable of proteolysing a foreign protein. To the stimulus exerted by the foreign protein these cells respond with the production of a new proteolytic enzyme manufactured by the mechanism of "enzymatic adaptation" (p 25). This new enzyme, however, has two disadvantages . (1) It can proteolyse only to the level of proteose, and (2) it can proteolyse only specific antigenic protein. The time required for the manufacture of this new toxic protease is designated as the period of sensitisation, and its length varies in various species of animals It also varies within the same species according to the type of antigen used for sensitisation.

In other words, in this mechanism of sensitisation, two independent and parallel processes take place

1 The lymphoid tissue along the paths of the penetration of antigen is stimulated to produce various antibodies (p 5)
2 The cells with inactive non-specific proteinases are stimulated to produce intracellular specific toxic proteases

The physiopathological process in which a normal organism is invaded continuously by various antigens in ordinary life may be represented by a simplified formula Such a formula illustrates the relationship between the invading antigen and the formation of antibody on the one hand, and the process of sensitisation on the other In this formula AG represents any antigen penetrating a normal organism, AB represents antibody produced locally in the lymphoid tissue around the portal of entry of the antigen, and the numbers attached to these symbols are arbitrary figures expressing the quantities of the operating antigen and antibody, A-Ar represents antigen-antibody reaction. Each line represents a further stage in the process.

PHYSIO-PATHOLOGICAL FORMULA OF IMMUNITY

Initial Stage—

AG 10 penetrates organism possessing AB 0, A-Ar 0; AG 10 spreads

AG 10 penetrates organism possessing AB 1; A-Ar+; AG 9 spreads

AG 10 penetreates organism possessing AB 2; A-Ar+; AG 8 spreads.

Further stages of antibody production and sensitisation, until

AG 10 penetrates organism possessing AB 10, A-Ar++; no AG spread

AG 10 penetrates organism possessing AB 15, A-Ar++; surplus AB 5.

(Description in text)

In the initial stage of AG 10 penetration the whole amount of AG 10 reaches the interior of the organism and various organs are penetrated by various quantities of AG, the cells with inefficient enzymes cannot proteolyse foreign protein and sensitisation supervenes. If all the cells of an organism possess efficient proteolytic enzymes, sensitisation does not follow despite the entry of antigen.

If during the period of sensitisation a sufficient quantity of *specific antibodies* becomes available, the anaphylactic reaction cannot supervene in spite of uninterrupted invasion by AG 10, because the barrier of antigen-antibody reaction prevents further entry of antigen into the sensitised cells. This state of physio-pathological equilibrium can be maintained only if the antigenic molecules are permanently neutralised by antigen-antibody reaction either in the regional lymph tissue or in the blood.

The production of antibodies in the organism invaded by antigen gives rise to three main varieties of the immunological response

1. The tissue which manufactures antibodies does not produce sufficient antibody to neutralise the whole

of the invading AG 10. As soon as sensitisation is established, the circulating antigenic protein entering sensitised cells precipitates various forms of anaphylactic reaction, this is the *primary anaphylactic reaction.*

2 The output of specific antibodies suffices to neutralise the whole of the AG 10. This means that antigenic molecules cannot pass the barrier of antigen-antibody reaction to reach sensitised cells (sensitised in the early stages of AG entry) The quantity of the available antibody is, however, inadequate to form a safety margin Should there be a rapid increase in antigen absorption or a decrease in the amount of antibody, the *state of equilibrium* may give place to *a secondary anaphylactic reaction*

3 The production of antibodies goes on until a surplus is obtained, by this action a powerful defence barrier is formed (AB 5 in the example quoted above) This safety margin successfully deals with an accidental *increase in the antigen or with a rapid decrease in the* titre of antibody This is the state of *active immunity*

The *state of equilibrium*, mentioned in (2), between the neutralised invasion of antigen and the co-existing state of sensitisation may, in suitable pathological conditions, initiate a manifest anaphylaxis For example, if production of antibodies has become suppressed by some exogenous poison such as barbiturates or iodides or by an infection, the balance between AG and AB becomes upset, this loss of balance leads to the invasion of the interior of the organism by some non-neutralised AG which initiates various anaphylactic reactions of *secondary type*

This formula gives a simplified picture of how various mechanisms may operate in the anaphylactic reaction Variations are possible, and numerous factors will influence the character of the response The quality of antigen has a decisive influence, thus if the antigenic protein is a virus or belongs to a bacterium, the response to such an antigen will be dominated and modified by the specificity of the "living antigen" In this case two (antigenic and virus or bacterio-toxic) pathogenetic factors of equal importance initiate at the same

time two different pathological mechanisms which are superimposed one upon the other. Other factors of equal importance in shaping anaphylactic response are the temperature and pH of the reaction. These may modify the yield of antibodies and change the velocity of the chemical and physico-chemical reactions.

Sensitisation by the Mechanism of Rapid Spread of Antigen.

In the mechanism of sensitisation induced by the rapid spreading of antigen via the blood stream (as in most cases of experimental anaphylaxis) the antigen reaches the interior of the organism without having made contact with the lymphoid tissue Antigen reaches all organs rapidly but the amount which actually enters the cells varies considerably because of the dilution. Those cells which are unable to proteolyse antigenic molecules manufacture a specific toxic protease in the manner described below Thus the difference between these two mechanisms of sensitisation lies in the rapidity of the antigen penetration, without initial contact being made with the regional lymph tissue

Lymphoid Tissue in Rapid Spread of Antigen.

In the hæmatogenous spread of antigen, the lymphoid tissue all over the body is invaded simultaneously with all other organs by a smaller amount of antigenic protein than it would have received by the (slow) lymphatic route , this difference is due to dilution and dissipation. Antigenic molecules are soon proteolysed by highly active non-specific trypsinases in normal organs as well as in the lymphatic tissue (Drinker *et al* , 1941 ; Fruton, 1946). Before being split the antigen stimulates lymphoid tissue to produce various specific antibodies. This short stimulation of lymphatic tissue may give two quantitatively different responses

1. The specific antibodies are produced and stored in lymphatic tissue until they are liberated into the body fluids by its disintegration in the course of normal ageing. The cells with a deficient proteolytic system have been sensitised by the same dose of antigen as entered the lymphoid tissue by hæmatogenous spread. In short, the sensitisation of the shock organs and the

production and commencing liberation of specific antibodies into the blood have occurred simultaneously. Neither process has interfered with the other.

2. If the lymphoid tissue has been unable to produce specific antibodies despite stimulation by antigen (*e g* , because of primary deficiency of lymphoid tissue, or because of too rapid disintegration of the antigenic molecules before the stimulus could be exerted), these antibodies do not appear in the body fluids The organs with the deficient enzyme system will, however, have become sensitised Thus the final state of affairs is that sensitisation has been established in the shock organs and that no specific antibodies are present in the organism

If the shock-dose of antigen invades the interior of the organism by both hæmatogenous and lymphatic routes, the sequence of events will differ as between (1) and (2) In the type of response shown under (1) some part or the whole of the dose of antigen may be neutralised by the circulating specific antibodies If only a part of the antigen is neutralised, the remaining active antigenic molecules will reach sensitised cells at the same time as they enter the lymphoid tissue. Antigenic molecules which make contact with lymphoid tissue are inactivated by one of the five forms of antigen-antibody reaction The part of the antigen which reaches sensitised cells precipitates a somewhat abortive form of anaphylactic reaction, since the shock-dose has been partly neutralised. Anaphylactic shock, like any violent stress, mobilises the pituitary-adrenal response. Lympholytic steroids are released and dissolve lymphoid tissue on a large scale. This large-scale release of antibodies (Dougherty *et al.*, 1944 *a*), as an emergency measure, comes, however, too late to neutralise the circulating antigen completely and thus prevent the anaphylactic shock. Hence, the finding of a high titre of specific antibodies in the blood, during the manifestations of shock or immediately after they have subsided, does not prove that they were present in equally high titre at the onset of anaphylactic shock. The biochemical events of the early stages of anaphylactic shock are of sufficient magnitude to precipitate the pituitary-adrenal response On the other hand, the velocity of release of

corticosteroids in the pituitary-adrenal response is so high (Vogt, 1950) that the effects of their lympholytic action may coincide with the manifestations of the later stages of the shock. The high titre of specific antibodies in these circumstances, therefore, does not prove that they were available in the same concentration at the onset of the shock and that by interaction with the shock-dose of antigen they precipitated the shock.

If the total shock-dose of antigen has been completely neutralised by circulating antibodies, the antigen will not reach the sensitised cells and anaphylactic shock cannot occur, *despite the injection of an apparently sufficient shock-dose.* An experimental animal showing such a lack of response is commonly regarded as *not sufficiently sensitised.* This certainly is not the case. The lack of anaphylactic response in these circumstances is caused by the high titre of circulating specific antibodies

In type (2) (in which specific antibodies have not been produced at all) the injection of a shock-dose is followed by a full-blown anaphylactic shock because the whole dose of antigen reaches the sensitised cells These animals are commonly regarded as *highly sensitised,* which they are not. The explanation of this *high sensitivity* lies in a complete lack of antibodies in the blood As a result the injected antigen has free access to the sensitised cells, which, however, are no more highly sensitised than those of type (1).

Anti-anaphylaxis.

The involution of the lymphoid tissue (with stored antibodies) following the stress of anaphylactic shock (p. 50) raises the titre of free proteolytic and non-proteolytic antibodies. The surplus of circulating antibodies is able to prevent a second anaphylactic shock should another dose of antigen be administered. This state, known as *anti-anaphylaxis* or *de-sensitisation,* develops if the animal survives the anaphylactic shock (Zunz *et al* , 1915) A similar procedure may be utilised for therapeutic purposes, except that full-blown anaphylactic shock should be avoided by the injection of a sub-toxic dose of antigen. A similar therapeutic measure is the production of non-specific shock by the injection of heterologous protein, colloidal sulphur, etc (p 44).

Passive Sensitisation or Passive Anaphylaxis.

Passive sensitisation or *passive anaphylaxis* is a condition in which a normal, never previously sensitised person or animal, if injected with the serum from an anaphylactic person or from an anaphylactic animal, may develop at the place of injection various forms of local reaction (wheal, urtica, etc) when forty-eight hours after the subcutaneous injection the specific antigen is administered into the general circulation An example of passive anaphylaxis is the Prausnitz-Kustner phenomenon (Prausnitz *et al*, 1921) Coca *et al* (1925) maintain that the antibody operating in the Prausnitz-Kustner phenomenon has a special character and they designated this antibody as *atopic reagin*

The enzymatic concept of anaphylaxis offers the following explanation of passive anaphylaxis The "toxic enzyme system" (nuclear factor plus molecule of specific antigen (for details see p 25)) formed in the sensitised cells is continuously excreted into the body fluids in various quantities, thus the serum obtained from an allergic subject possesses a certain amount of this *toxic enzyme system* as free protein molecules So long as these large molecules remain in the infiltrated tissue (they remain there by force of adhesion) the local enzymatic set-up is identical with that of the donor-sensitised organism There are, however, the following differences between the donor (sensitised) and the recipient (non-sensitised) organism

1. In the sensitised organism the *toxic enzyme system* is distributed all over the organism, whereas in the recipient organism the *toxic enzyme system* is only locally accumulated
2. In the donor organism the *toxic enzyme system* persists permanently with some fluctuations in its concentration, whereas in the recipient organism the locally accumulated toxic enzymes disappear in a short time as the result of normal proteolysis.

Anamnestic Reaction.

Anamnestic reaction (Greek: ἀνάμνησις=recollection) is a reaction to the parenteral administration of a heterologous protein in which various antibodies (not related to the injected protein) appear in the body fluids The enzymatic concept of

anaphylaxis explains this reaction in the following way: Injected molecules of the heterologous protein release the pituitary-adrenal response and liberated lympholytic corticosteroids produce rapid involution of the lymphoid tissue. Those antibodies whose production was induced sometime in the past, and which have been stored in the lymphoid tissue, are released into the body fluids in the course of the disintegration of the lymphoid tissue Therefore any factor which is able to release the pituitary-adrenal response is apt to induce anamnestic reaction

Histo-chemical and Sub-microscopical Structure of the Cell.

Before entering into a discussion of the altered chemistry of the cells and body fluids found in anaphylactic reactions it would be well to summarise some of the established facts of the present-day knowledge of sub-microscopical cellular structure. The next few paragraphs are based mainly on three sources: Frey-Wyssling's (1953) monograph on sub-microscopical biology of the cytoplasm, Monnè's (1948) review of cellular enzymatic structure, and "The Enzymes" by Sumner and Myrback (1950). The data will be presented in summary form; they will serve as a framework and basis for a more detailed review of cellular changes found in anaphylaxis and in allergy.

The cytoplasm of a living cell consists of semi-liquid matter composed of proteins, lipoids, carbohydrates, electrolytes, etc., dissolved or suspended in water The framework of this mass is built mainly of proteins whose molecules possess a fibrillar structure and form a fine network. A stream of water (water plus substances dissolved in it is called by some workers enchylema, Monnè, 1948) constantly circulates in the interfibrillar canals thus formed and contacts various parts of the cell by means of chemical, physical, and physico-chemical interactions. The protein fibrils are formed by a variable number of polypeptide chains linked with each other by the end- or side-groups of the amino acids from which the chains are built. This linkage is formed either by direct interaction of their side- or end-groups, or by the formation of salts (bridge formation) by various polyvalent ions (Fig. 1, A, B, and C).

The cortical part of the cell (called also the cell membrane) is composed mainly of lipoids whose rod-shaped molecules are in some places pierced by protein molecules The polypeptide

chains of these protein molecules are continuously coiling and uncoiling, shrinking and swelling, shortening and elongating. In so doing they cause "periodical fenestration" in the cell

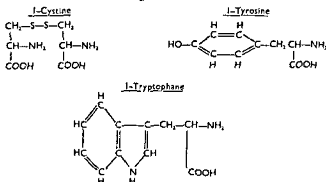


FIG. 1A

Examples of three main types of amino acids (aliphatic, aromatic, and hetero-cyclic) which build polypeptide chains

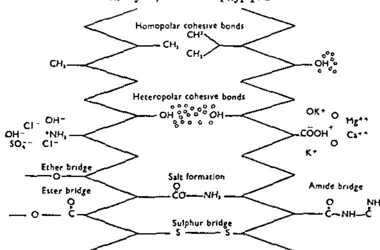


FIG 1B

Diagrammatic representation of junction between neighbouring polypeptide chains in the cytoplasm, O = water molecules. (From A. Frey-Wyssling, "Submicroscopic Morphology of Protoplasm," Second English Edition, 1953. Elsevier Publishing Co. Inc, New York and Amsterdam)

membrane and thus permit a foreign molecule, whether protein or not, to enter the cell and induce various chemical or physico-chemical reactions such as *enzymatic adaptation*, inactivation of cellular lipoids, etc (Fig. 1, A, B, and C).

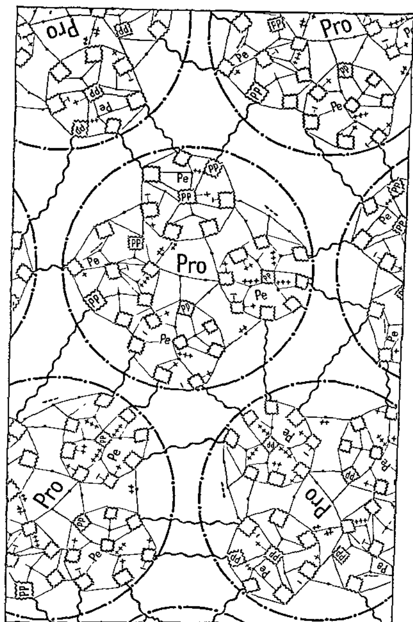


Fig. 1c

Model of a molecule of a simple protein of which eight molecules of proteose *are seen*

—— represents linkage between polypeptide molecules (PP); this linkage is hydrolysed by exo-peptidases (Smith, 1952)

—·—·— represents linkage between peptone molecules (Pe) by polyvalent ions, this linkage is hydrolysed by endopeptidases (proteases)

~~~~ represents linkage between molecules of proteoses (Pro), this linkage is hydrolysed also by endopeptidases.
~~~~

Antigenic Conditioning of Proteins.

For a foreign particle to enter the cell certain conditions must be fulfilled; a particle of appropriate size must be adjacent to an open cortical fenestra and a force must be available to drive it through the cortex into the interior. Two points in this statement require to be discussed in greater detail

1. The size of the particle
2. The force of *suction* which actively shifts the particle into the interior

1 The importance of the size of the penetrating particles must be strongly emphasised because size is one of the factors determining the antigenicity of a given protein.

Foreign protein molecules, whether they have originated outside or inside the organism, are disseminated all over the organism by the blood and body fluids (Coons *et al.*, 1951) Protein molecules of size larger than that of the cortical fenestra of the host-cell cannot penetrate the cellular interior, for the fenestræ form the sole means of entry. Therefore these proteins cannot exercise any antigenic action, despite the fact that they possess all the other characteristics of an antigenic protein

The importance of molecular size in the determination of the antigenic potency of a given protein has been stressed by many investigators (Neuvil *et al*, 1946, Loiseleur *et al*, 1947, Loiseleur, 1950, and others), their statement that the antigenicity of a protein varies inversely with its molecular size is consistent with the theory described above, *i e*, that antigenic molecules enter the cell through the fenestræ

The difference in the molecular size of various proteins also explains the fact that protein which is highly antigenic when applied to one species may show no antigenicity when applied to another. Different species have proteins of different molecular size and that confers specificity on their proteins; hence their cell fenestræ are also of different sizes

2. The force which moves the foreign particle from the outside to the inside of a cell through the fenestræ in the cortex may be described as "suction." This suction is due to periodical movements of the protein molecules in the cortical canals of the cell. Protein molecules periodically shrinking and extending in the cortical canals exercise movements which

may be compared with the piston moving in the cylinder whereby the fuel mixture is sucked into the cylinder of a motor engine. By this mechanism the foreign particle is passively *aspirated* into the interior of the cell.

Antigenic potency depends also on the chemical and physico-chemical properties of the protein. For example, protein molecules deprived of their lipoid conjugation acquire strong antigenicity, whereas if the lipoid-free protein moiety is covered with lipoids such as sodium oleate it loses some of its antigenicity (Jobling *et al*, 1915 *a*) These alterations in antigenic power, which are related to the presence of the lipoids in the protein molecules, depend on a twofold action of the lipoid-free proteins in the interior of the invaded cell:

1 By surface-acting forces they remove the cellular lipoids from the lipo-protein molecules of structural proteins (p 55)
2 They augment the existing toxic enzymatic system, built in the course of sensitisation (p. 28); by this action they initiate toxic proteolysis by specific proteases

In short, a *protein, if it is to have antigenic potency*, must fulfil the following three conditions.

1 The size of its molecules must be smaller than those of the structural proteins of the invaded cell
2 Its molecules must escape proteolysis and be brought in unsplit form to the surface of cells possessing an inadequate enzymatic system, they must be placed sufficiently close to an open fenestra to be sucked through it
3 Its chemical or physico-chemical structure must be such as to induce the formation of a toxic enzyme system and to initiate its activity in the sensitised cells

Terminology and Classification of Proteinases.

The term *proteinases* is applied to all types of proteolytic enzymes The terminology of the intracellular proteinases accepted in this essay is based principally on Bergmann's classification (1942). His earlier work with Fruton and Irving (1941) provided him with the framework of this classification.

He distinguishes, amongst others, two types of Cathepsin (intracellular proteinases) which he extracted from various bovine and porcine organs. Their proteolytic action on synthetic substrates resembles either

1. That of gastric pepsin, and these he called *pepsinases* or *Cathepsin I*, or
2. That of intestinal trypsin, and these he called *trypsinases* or *Cathepsin II*.

However, he found differences between the action of intracellular proteinases and that of the proteolytic enzymes of the alimentary canal. For example, the optimal *pH* for the proteolytic action of gastric pepsin is 1·5 to 2, whereas for beef spleen pepsinase it is 5 6 , for intestinal trypsin it is 7·8 and for beef spleen trypsinase it is 4 9. This demonstration of two different intracellular proteinases, each splitting proteins to a different level of degradation, is of fundamental importance to the enzymatic concept of anaphylaxis and allergy

The terms *toxic proteinase* and *toxic enzyme system* are used *in this essay for those enzymes which by their hydrolytic* activity reduce the protein molecules to the level of toxic catabolites For example, a proteinase which splits protein molecules to the level of proteose and which liberates proteoses intracellularly or extracellularly is called a toxic protease

Specificity of Intracellular Proteinases.

Intracellular proteinases were regarded at one time as specific, and *this belief formed the basis for the Abderhalden* reaction (Abderhalden *et al* , 1909, 1910, *a* and *b*, 1911). Jobling with his co-workers (1915 *a*) proved this theory to be not entirely correct They separated various proteinases from serum and demonstrated that the tryptic proteinases possessed the capacity to proteolyse any protein provided that the lipoid "anti-ferments" of the serum had been removed The present view is that only some proteinases are specific.

The proteolytic enzymes classified in this essay as trypsinases or Cathepsin II are non-specific in character and are normally abundant in all living tissues Lymphatic tissue is particularly well provided with highly active, non-specific proteinases (Drinker *et al.*, 1941 ; Fruton, 1946).

A very brief summary of two theories, illustrating the

specificity of enzyme-substrate proteolysis, is presented here. These are .

1. *The binding planes* theory (Bergmann, 1936).

2. *The bridge formation* theory (Smith, 1948, *a* and *b*).

1. *The binding planes* theory holds that enzyme and substrate molecules cannot interact unless the configuration of atoms and atom groups located on the interacting planes is such that they fit spatially into one another. In other words, the specific combination between enzyme and substrate depends on the pattern in space of the interacting molecules

2 *The bridge formation* theory holds that the molecules of

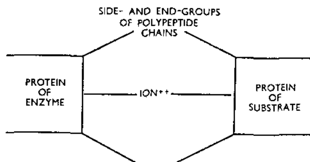


FIG. 2
Description in text

an enzyme and a substrate cannot start the enzymatic process until the reaction between the end- or side-groups of the interacting molecules is completed. One or more of the end- or side-groups cannot react with the corresponding group of the opposite molecule unless *a bridge* is formed between them by one polyvalent metal ion Thus the required ionic bridge enables the initiation of the enzymatic process, provided the polyvalent ion itself can react with both enzyme and substrate molecules In this way the polyvalent ion selects chemically those molecules which can initiate the enzymatic process By this action the ionic bridge confers the specificity upon the enzyme and protein This theory is presented above in *diagrammatic form in Fig. 2*

The distinction between specific proteolytic enzyme and specific proteolytic antibody has been discussed on page 8.

Reorganisation or Adaptation of Intracellular Proteinases.

Sufficient evidence has been adduced that an unsplit protein molecule or a colloidal particle of suitable size can enter a normal cell (Monnè, 1948), this penetration takes place during the periodical fenestration of the cortical part of the living cell (p. 18) Protein molecules, marked with fluorescent dye, were demonstrated by Coons *et al* (1951) in the cytoplasm and nuclei of cells which they had entered by this route The foreign protein molecules remaining intact inside the cell upset the intracellular equilibrium by their chemical and physico-chemical actions, thus delivering a toxic stimulus to the nucleus In response to this stimulation the nucleus produces an *organising factor* for a new enzyme, and this nuclear element enters the cytoplasm (Monnè, 1948) The nuclear *organising factor* may be looked upon as a gene which, having reached the cytoplasm as a *cytoplasmatic gene* (Spiegelman *et al*, 1946), interacts with the foreign protein molecule and so creates a new enzymatic system This process, leading to the formation of a new proteinase, may be set in motion if the existing intra-cellular proteinases are unable to split the foreign protein (see next paragraph). The formation of a new proteinase in these circumstances must be regarded as a counter-measure taken by the nucleus against the toxic action of the foreign molecules remaining unsplit inside the cytoplasm. If the toxic impact made by the foreign molecules on the biochemistry of the nucleus is deep enough, or if the constitutional biochemistry of the nucleus is such that it cannot form a new normal enzyme in response to the toxic stimulation, then the nucleus may manufacture a pathological or toxic proteinase The toxicity of the new enzymatic system may be characterised by only partial proteolysis and by action solely on molecules of the same protein which participated in the formation of the toxic enzyme system, *i.e*, this toxic enzyme may be specific.

The reaction of the invaded cell to a foreign protein may take a different turn. The new *adaptive enzyme* may be able to carry out complete hydrolysis of the invaded protein. Such an enzyme, according to Bergmann's suggestion (1942), may, in suitable conditions, be bi-phasic. In the catabolic phase the large protein molecule is split to its primitive components—amino acids. In the anabolic phase of the same proteinase, the

re-synthesis of the protein molecule may be accomplished. If the new *adaptive enzyme* has catabolised the intruding protein it may, in its anabolic phase, re-synthesise it. Ultimately this replica of the foreign protein may be incorporated into the structural proteins of the primarily invaded cell.

The future of these cells is influenced by many factors. They have acquired at least two new features, namely, a new proteolytic enzyme and a new structural protein. These two characteristics completely alter the nature of these cells. The character of these cellular units will entirely depend on:

(1) The character of the *adaptive enzyme*;
(2) The character of the reconstituted protein.

1. If the anabolic and catabolic activities of the *adaptive enzyme* are well balanced, and if the reconstituted protein molecules have no pathological features, the cellular metabolism will be little altered. If catabolic activity predominates in the cellular metabolism, these cells soon die out and the whole incident is closed. More pregnant in consequences is the alternative in which the anabolic phase of the *adaptive enzyme* predominates in the metabolism of the affected cells: the subsequent pathology will depend to a great extent on the type of reconstituted protein.

2. The primitive catabolites derived from hydrolysis of the foreign protein are at first the sole protein material for the reconstitution of its molecule by the anabolic phase of the *adaptive enzyme.* A further supply of the required material is later derived from the organism's own resources. The newly synthesised protein molecule is a replica of the invading protein since it was built by the *adaptive enzyme*. This replica next becomes incorporated into the structural proteins of the invaded cell. Thus the foreign protein is incorporated into the organism's own structural proteins, by this action a fundamental and deeply rooted alteration has been implanted in the affected cells.

If the character of the *adaptive enzyme* is of anabolic type and the self-renewing protein possesses antigenic power, *i.e.*, gives rise to toxic protease, a vicious circle may develop.

The invading protein may be pathogenic not only by virtue of its antigenic power, for example, it may be a virus protein. If so, the vicious circle is doubly harmful, since the reconstituted

protein has two distinct pathological effects. But this aspect of the problem cannot be discussed further in this essay, for such discussion would lead us into the vast fields of inflammatory and neoplastic pathology.

The process of new formation or reorganisation of the existing intracellular enzymes under the action of a new substrate is called by some enzymologists *enzymatic adaptation* (Karström, 1938, Spiegelman, 1950, and others). This theory is built on observations made on lower organisms, but there is a strong suggestion that the same process may take place also in higher organisms (Burnet *et al.*, 1949).

This concept is, however, not universally accepted in its original form Those who object to it argue that the new substrate (*i e*, the penetrating antigen) must be chemically closely related to the existing substrate (*i.e.*, the cellular structural proteins) for *adaptation* to be possible This argument implies that the new substrate stimulates the existing enzymatic system in such a way as to bring out the activity of an enzyme already present in the cell (Sevag, 1946)

Toxicity of Enzymes.

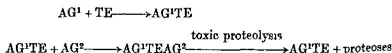
Some enzymes possess biological toxicity which they exercise in various pharmacological ways (Marrack, 1950) Some of the toxicity of the toxic proteinases is inflicted by the end products of the hydrolysis carried out by them (Peters, 1952 *a*) For example, proteoses resulting from this proteolysis may appear in the tissues or in the body fluids and be highly toxic, as, for example, in anaphylactic shock, the protease responsible has acquired the features of a toxic enzyme.

Peterman (1942) has suggested that there may be two types of enzymatic proteolysis · (1) *the all or none* type, in which large protein molecules are broken down rapidly to small dialysable fragments, (2) *the piecemeal* type, in which highly dialysable fragments are split off, leaving heavy particles but no unchanged protein The pattern of *piecemeal* hydrolysis may be the one which occurs in the anaphylactic reaction Partially altered protein molecules which have been reduced in size by the splitting-off of a certain number of light dialysable fragments (amino acids) are, of course, proteoses—the first breakdown products of proteins In a later discussion it will be pointed out (p. 39) that Menkin (1950) was able

to reproduce some of the manifestations of anaphylaxis by parenteral injection in non-sensitised animals of substances which he had isolated from inflammatory exudates; these isolated substances were products of high-level proteolytic degradation. It is therefore reasonable to assume that the toxic substances isolated by Menkin are identical with the proteoses liberated by *toxic proteases* *Similar toxic proteolysis* may occur in other types of inflammation

The formation of the specific toxic enzymatic system takes place during the period of sensitisation which runs the following course the nucleus invaded by the antigenic molecules is stimulated by the toxic action of the unsplit protein (Branchet, 1950), this results in the formation of an *organising factor* for the formation of a new enzyme (Monnè, 1948) The *organising factor*, when it enters the cytoplasm, reacts with other molecules of the same (sensitising) dose of antigen by the mechanism of *couple reaction* The newly formed toxic enzymatic system can start proteolysis only when another molecule of the same protein augments the *coupled* enzymatic system (Bergmann, 1942), this augmentation takes place when antigenic molecules enter the sensitised cells, as, for example, during the administration of the shock-dose.

The process of sensitisation and anaphylactic response can be presented in the following formula



AG^1 represents unsplit antigenic molecules of the sensitising dose in the interior of a cell to be sensitised, AG^2 represents unsplit antigenic molecules of the shock-dose; TE represents toxic enzyme inside a sensitised cell, AG^1TE represents toxic enzyme system inside and outside the sensitised cells, AG^1TEAG^2 represents augmented toxic enzyme system which immediately splits the augmenting antigenic molecule (AG^2) to the level of various proteoses At the end of this hydrolysis toxic enzyme system (AG^1TE) remains intact inside the sensitised cell ready to be augmented again if another shock-dose of specific antigen enters the sensitised cell.

Ability to produce non-specific proteinases and inability

to form toxic proteases appears to be, in some cases, organ and species specific. For example, the connective tissue surrounding the bronchioles in guinea-pigs responds to the injection of a toxic dose of specific antigen by forming gross œdema (Warren *et al.*, 1948) ; hence, according to the enzymatic concept of anaphylaxis, the cells of the connective tissue around the bronchioles in guinea-pigs form toxic specific protease during the course of sensitisation Why only certain tissues respond to antigenic stimulation by forming specific proteases in species specific anaphylaxis is difficult to say In the case of hereditary sensitivity the root of this inability lies probably in a genetic deficiency of the biochemical constitution of the nucleus (Bourne, 1951), whereas in the case of acquired sensitivity the inability may be caused either by a constitutional debility of the enzymatic system (in the given organ or given species) or by the ageing of cellular proteolytic enzymes in the course of normal or pathological *wear and tear*

Protein Catabolism seen in the Sub-microscopical Structure of the Cell.

The cytoplasmic proteolytic enzymes are proteins whose fibril-like molecules are formed of polypeptide chains just as are those of substrate proteins The enzymatic proteins are, however, separated from the cytoplasmic substrate proteins by an insulating barrier of lipoids as shown in Fig 3 This insulating action of lipoids depends on a homopolar cohesive linkage between the end- and side-groups of the polypeptide chains of the substrate and those of the enzyme proteins This blocking action of lipoids has been called by various authors the *antiferment potency* (Jobling *et al*, 1915*a*) Any action which can disrupt this intracellular lipo-protein linkage is apt to start proteolysis. The velocity and co-ordination of the proteolysis depends on the manner in which the lipoid molecules are removed from the lipo-protein conjugation. For example, surface-acting substances, such as molecules of proteins or proteoses (or any other colloidal particles) can initiate proteolysis by rapid alteration of the dispersion phase of lipoids. Colloidal molecules reaching the interior of a cell may start a chaotic proteolysis in this way. If the cells involved possess a specific toxic enzymatic system (*i e*, if they are

sensitised), the specific antigenic molecules can initiate an uncontrolled toxic proteolysis by the mechanism described on page 28.

The chemical reactions resulting from the substrate-enzyme union lead to the removal of the peripheral amino acids or polypeptide chains from the substrate proteins (Fig. 1c). Temporary inactivation of the cellular lipoids may also be achieved through other mechanisms, as, for example, by a purely chemical action (p. 53). It has been proved (Jobling *et al.*, 1914) that the lipoid molecules can exercise their blocking action only if they possess unsaturated carbon atoms in their fatty acids. The saturation of these carbon atoms by various chemical methods (p. 108) may be another mechanism by which intracellular lipoids are inactivated It is not the mechanism, however, by which proteoses inactivate lipoids. These operate in all probability by a physico-chemical means, *e g.*, by altering the dispersion phase of the lipoid molecules (Jobling *et al.*, 1915 *a*). For further discussion on this problem see Chapter II

ANAPHYLACTIC SHOCK AND THE ANAPHYLACTIC REACTION

Peptone Shock.

It has long been known that so-called peptone shock and anaphylactic shock are two identical reactions so far as their manifestations are concerned (Biedl and Kraus, 1909; and others). The resemblance of these two syndromes is so marked that most of the early work concerned with the fundamental problems of anaphylaxis were studied on peptone shock. The main argument against the peptone-shock theory of anaphylaxis was that blood withdrawn from a dog in anaphylactic shock contained no transferable toxic agent (peptone) by which peptone shock could be reproduced in another non-sensitised dog (Weil, 1917).

Grosjean in 1892 found that intravenous injection of Witte's peptone in a dosage of 0·15 to 0·30 gm. per kilo into a normal dog produces a reaction shown by later workers to be characteristic of anaphylactic shock (Table 1). The basic difference between these two syndromes is as follows · in anaphylactic

shock the peptone molecules (or any other proteoses) are liberated inside a previously *sensitised cell*, whereas in peptone shock the peptone molecules *invade a non-sensitised cell* from outside. In order to produce peptone shock in a non-sensitised dog one must inject a relatively high dose of peptone, because

TABLE 1

Comparison of the Manifestations of Anaphylactic and Peptone Shock with their References

Manifestation.	Anaphylactic Shock.	Peptone Shock.
Hyperheparinæmia	Jaques *et al*, 1940	Quick, 1936
Hyperhistaminæmia	Code, 1939	Dale, 1929
Leucopenia	Widal *et al.*, 1921	Widal *et al*, 1921
Thrombocytopenia	Dragstedt, 1941	Rocha è Silva, 1946
Increased titre of non-specific proteinase in blood	Jobling *et al*, 1915 *c*	Jobling *et al*, 1915 *c*
Increased titre of protease in blood	Pfeiffer, 1915	.
Increased NPN in blood	Jobling *et al*, 1915 *c*	Jobling *et al*, 1915 *d*
Hyperglycæmia	*Zunz, 1924*	*Zunz, 1924*
Hypoproteinæmia	Petersen *et al*, 1923	Petersen *et al*, 1923
Increased proteins in lymph	Petersen *et al*, 1923	Petersen *et al*, 1923
Increased lymph flow	Petersen *et al*, 1923	Heidenhain, 1891
RBC 's in lymph	Petersen *et al*, 1923	Heidenhain, 1891
Increased permeability of capillaries	Calvary, 1911	Calvary, 1911
Decrease of plasma volume with hæmoconcentration	Simonds, 1923	Simonds, 1923
Fall in blood-pressure	Simonds, 1923	Simonds, 1923

(1) the dose is diluted by the body fluids and (2) the amount of peptone which reaches the interior of the cell must be well above the proteolytic capacity of the invaded cell

Injected peptone molecules penetrate all organs, and those cells which possess highly active intracellular non-specific proteinases split the peptone molecules immediately to the level of non-toxic catabolites. Cells possessing insufficiently active proteinases cannot proteolyse the whole shock-dose of

peptone; the portion which escapes proteolysis inflicts its toxic action upon the intracellular lipoids in a manner resembling that of the proteoses produced intracellularly during the anaphylactic reaction (see next paragraph) Peptone in a dosage lower than that required for shock is immediately proteolysed and there is no surplus of peptone left to initiate rapid and inco-ordinated intracellular proteolysis. This point is worth stressing, because it explains why in the blood of a dog in anaphylactic shock Weil (1917) could not discover any transferable agent capable of reproducing peptone shock in another non-sensitised dog

Anaphylactic Shock.

Anaphylactic shock and the anaphylactic reaction are phenomena which differ from each other only quantitatively. Both take place in sensitised cells, both result from the invasion of the interior of sensitised cells by antigen, and in both the intracellular specific toxic proteases hydrolyse the antigenic protein to the level of peptone-like proteoses The main difference lies in the amount of antigenic protein penetrating the sensitised cells, and in the rate of its penetration

By detailed analysis of the histo-chemical events involved in anaphylactic shock, four stages of shock may be distinguished This separation into stages is somewhat arbitrary because each phase of the reaction runs uninterruptedly into the next with a very high velocity. However, for purely practical purposes of presentation of the chemical events, a slow-motion picture of the biochemical sequence in anaphylactic shock has been formulated Its value is solely descriptive

First Stage of Anaphylactic Shock

The shock-dose of antigen enters sensitised cells which already contain a toxic enzymatic system as a result of sensitisation The antigenic molecules augment this enzymatic system and thereby set in motion the toxic proteolysis of those antigenic molecules which have augmented the enzymatic system with the production of free proteose molecules in the cytoplasm At the end of this hydrolysis the toxic enzyme system is ready again to be augmented (and start proteolysis) by another molecule of antigen if it is available in the cell

(p 28), the turnover of this reaction will depend on the dosage of the applied shock-dose of antigen.

Second Stage of Anaphylactic Shock

Proteose molecules which have been born in the cells disrupt the lipo-protein molecules by altering the dispersion phase of the lipoid component The cytoplasmic structural proteins, deprived rapidly of their lipoid protection, are chaotically exposed to the hydrolytic action of the cellular non-specific proteinases which have been activated by the same action (lipo-protein disruption) Uncontrolled proteolysis of the structural proteins of the sensitised cells begins in this way and eventually leads to the disruption of the cellular structure and to the release of the cellular enzymes and toxic catabolites into the body fluids

These first two stages may be regarded as the intrinsic phase of anaphylactic shock because they occur inside the sensitised cells

Third Stage of Anaphylactic Shock

The toxin released into the blood during the first two stages of anaphylactic shock exerts its pharmacological actions in organs hitherto unaffected and so gives rise to the various constitutional manifestations of anaphylactic shock The third stage may therefore be looked upon as the extrinsic phase of anaphylactic shock

Fourth Stage of Anaphylactic Shock

In this stage the final issue of anaphylactic shock takes place either recovery or death.

In the recovery period the organism may acquire (1) an immunity against further invasion by the specific antigen of the organism Such immunity is due to a massive liberation of specific and non-specific antibodies by lymphoid tissue as a result of the lympholytic action of the pituitary-adrenal response (p. 49), (2) pathological lesions in the shock organs or in the organs not primarily involved in the anaphylactic reaction (ectopic lesions) : both of these changes may ultimately heal or may establish (3) secondary chronic anaphylactic conditions (see below).

Chronic Anaphylaxis.

The severity of the anaphylactic shock depends on the amount of proteose released in the cells. This in its turn depends on two conditions :

1. On the amount of the antigenic protein which has entered the cells
2. On the kinetics of the intracellular toxic proteases and non-specific proteinases

If the velocity of the protease hydrolysis is rapid enough and if the amount of antigenic protein is adequate, a full-blown anaphylactic shock results If, however, only a small amount of antigenic protein has entered the cells and this has been peptonised, the amount of released proteose is also small and the damage done to the lipo-protein structure is relatively slight. The symptomatology of this mild shock is therefore less dramatic But this mild degree of shock, if continually repeated, is apt to precipitate cellular alterations (see below) of sufficient magnitude to produce a pathological response called **primary chronic anaphylaxis or the primary chronic allergic condition.** If an acute shock precedes the onset of the chronic manifestations, the chronic condition is called **secondary chronic anaphylaxis or the secondary chronic allergic condition.** The acute develops into and merges with the chronic stage.

The manifestations of both types of chronic anaphylaxis may be localised either in the shock organs or in the distant tissues (ectopic reactions) or in both The description of the local and constitutional manifestations of the anaphylactic reaction is deferred to the next few paragraphs

Heparin in Anaphylactic Reaction.

Heparin is present in all living cells in variable amounts, either free or conjugated with proteins (Chargaff, 1941). If a protein salt of heparin is exposed to the proteolytic action of intracellular enzymes, the heparin molecule may be split off. Free heparin is liberated in and eliminated from the affected cell. The remaining cellular proteins, deprived of heparin, undergo reversible gelation (Fisher, 1931). This change in the physico-chemical structure of the substrate proteins may

influence the affected cell in various ways, a mobile cell, for example, may be immobilised An example of such action is the arrest of moving eosinophils in the lymphatic barrier in the lamina propria of the intestinal wall (p. 74) If the amount of the tissue involved in the anaphylactic reaction is great enough, so much heparin may be released that, entering the blood, it will cause its incoagulability. This actually occurs in the early stage of both peptone shock (Quick, 1936) and anaphylactic shock (Jaques *et al*, 1940) In the stage of recovery from the shock, heparin re-enters the interior of the gelated cells and restores the semi-liquid state of their cytoplasmatic proteins (Fisher, 1931), if the cells were immobilised, it restores their mobility (Godlowski, 1952 *a*). The re-entrance of heparin into the cells leads to rapid reduction of the heparin level in the blood This in turn may create conditions favourable for spontaneous clotting, especially where there is a physiological slowing of blood flow, as, for example, at the junction of a collateral with a main vein (*e g.*, the junction of saphenous and femoral veins). This fall of heparin concentration may explain the tendency to thrombotic accidents in the clinical conditions associated with various types of anaphylaxis (Gauthier-Villars, 1951) Another consequence of the fall in heparin concentration is capillary thrombosis with some extension proximally and distally into arterioles and venules. The mechanism of this capillary thrombosis may be as follows in the recovery stage from acute or chronic anaphylactic shock, the focus involved in the anaphylactic reaction rapidly absorbs heparin from the passing blood, and so promotes clotting in the capillaries of the affected tissue This in turn causes an acute ischæmia of the tissue involved in the anaphylactic reaction, leading finally to *ischæmic necrosis*

The release of heparin and its sequelæ are the mere consequences of the intracellular proteolysis initiated by the inactivation of the intracellular lipoids by proteose molecules. The release of heparin is thus secondary, having no direct causal connection with the primary mechanism of the anaphylactic reaction, therefore heparin injected in large doses should not influence the initiation of shock This conclusion was confirmed by an experiment in which the injection of a very large dose of heparin (2,000 units per kilo) into sensitised rabbits and guinea-pigs did not lessen the lethal

effect of an injection of antigen thirty minutes later (Godlowski, unpublished data)

Histamine in the Anaphylactic Reaction.

If the uncontrolled catabolic activity of the anaphylactic reaction splits off the amino acid histidine from the polypeptide chain it may be rapidly converted into histamine by histidine-decarboxylase (Holtz, 1937, Blaschko, 1945) Histamine may be released also by a different mechanism In conjugated form it exists in almost all tissues (Gaddum, 1948) In the course of toxic proteolysis such conjugated histamine may be liberated in the cells, where it exerts an intrinsic action, and may escape into the body fluids, where it exerts its extrinsic action, precipitating a great variety of manifestations in various tissues By its extrinsic action it is responsible for most of the dramatic symptoms of anaphylactic shock (Gaddum, 1948) and of *syndromes resembling anaphylactic shock* (p 40) Its powerful action on epithelium and capillaries, on the smooth muscles of various organs and on the secretory action of various glands, is the chief agent responsible for most of the symptoms of shock, so that at one time the anaphylactic reaction was regarded as histamine poisoning (Dale *et al*, 1910-11, Dale, 1929, Schild, 1939, Dragstedt, 1941, and others). The violent symptoms of the anaphylactic shock produced mainly by the release of histamine involve the pituitary-adrenal response (Sayers and Sayers, 1947) with its own train of effects (for details see " Hormonal Involvement in Anaphylaxis ")

Heparin and histamine should therefore not be regarded as taking an integral part in the anaphylactic reaction, they may or may not do so The liberation of these substances will depend on the chemical constitutions of the split proteins and on the kinetics of the available enzymes in the affected tissues If the proteins involved in the toxic proteolysis contain a sufficient quantity of heparin salts, then heparin may reach the body fluids in detectable amounts If the affected proteins contain enough histidine and if this histidine is set free by uncontrolled proteolysis, then *histidine-decarboxylase, if available*, may convert it into histamine. But if histaminase is present in sufficient concentration at the place of the reaction it may destroy the released histamine and so prevent it from exerting any extrinsic action (Gaddum, 1951). In other words,

an anaphylactic reaction may take place without the release of either heparin or of histamine or of both. The leading symptoms of anaphylaxis may therefore vary from case to case although the principal pathogenetic mechanism remains the same. They may vary in different species and in various syndromes within the same species

Proteolytic Enzymes and Proteolytic Catabolites in the Anaphylactic Reaction.

Human and dog sera normally contain tryptic enzymes whose activity is blocked by the blood lipoids. When the dispersion phase of these lipoids is altered, the proteolytic enzymes become active *in vivo* and *in vitro* against both homologous and heterologous proteins (Jobling *et al*, 1914) Extraction of a normal serum with chloroform activates these enzymes, when the lipoids extracted by the chloroform are added to the treated serum its tryptic enzymes become inactive again. Similar effects may be obtained by extracting with other fat solvents, by saturating the unsaturated carbon atoms of their fatty acids, or by altering the dispersion phase by means of surface-acting substances (Jobling *et al*, 1915 c, see also p 108) A similar mechanism of proteolytic activation may operate in intracellular and extracellular proteolysis. Thus proteolysis in the blood and in the tissues is controlled by the availability of active lipoids for the insulation of the substrate proteins against normally present non-specific proteolytic enzymes.

In the anaphylactic reaction an alteration occurs in at least three enzymes in the blood

1 A quantitative change in the titre of non-specific proteinases.
2 The appearance of toxic proteases
3. An increased titre of serum esterase

1. During anaphylactic shock the non-specific proteolytic power of the serum rises rapidly This leads to a significant elevation of non-protein nitrogen (NPN) in the blood *in vivo* (Abderhalden *et al*, 1911; Jobling *et al*, 1915 c, and others). Increased tryptic activity of the blood obtained from an anaphylactic dog has also been demonstrated *in vitro*

(Abderhalden *et al*, 1911, Zunz *et al.*, 1915; Pfeiffer *et al.*, 1915). The rise of the tryptic power of the serum varies with the severity and the duration of the anaphylactic shock (Jobling *et al*, 1915*c*) Since the increase of NPN in the blood of an anaphylactic dog exceeded the total N content of the applied shock-dose of antigenic protein, Jobling and his co-workers (1915*c*) rightly concluded that the excess of NPN in the blood is derived in these circumstances from the hydrolysis of the organism's own proteins In other words, during the anaphylactic reaction a rapid toxic proteolysis of antigen and increased normal proteolysis of the organism's own proteins elevate *the NPN in the blood*. on *the other hand*, the increased and accelerated non-specific proteolysis depletes the organism's stores of proteins

The increased proteolysis in anaphylaxis is caused by two factors ·

(*a*) Inactivation of lipoids which protect substrate proteins.
(*b*) Increased output of non-specific proteinases.

(*a*) The inactivation of lipoids in intracellular and extracellular lipo-protein molecules is due to the release into the body fluids of free molecules of various proteoses during the second stage of anaphylactic shock, the proteose molecules disrupt the lipo-protein linkage by surface-acting forces (p 55)

(*b*) The elevated titre of non-specific proteinases is due to the massive dissolution of lymphoid tissue caused by the lympholytic steroids released during the pituitary-adrenal response This is put into operation by the stress of the anaphylactic shock itself.

2 The appearance of toxic proteases in the blood of a sensitised dog, and the rapid increase in its titre during anaphylactic shock, has been independently established in various laboratories (Vaughan *et al.*, 1913, Jobling *et al.*, 1915*c*, Zunz *et al*, 1915, Pfeiffer, 1915, and others). The titre of the serum proteases gradually rises during the period of sensitisation and reaches its peak at about the fifteenth day. According to the suggestion of Jobling *et al.* (1915c) *the initiation* of protease-hydrolysis in anaphylactic shock is made by the inactivation of the blood and tissue lipoids which block its action.

The toxic proteolysis carried out by various proteases takes

place mainly in the sensitised cells but partly also in the blood during the second stage of anaphylactic shock (p 33) The toxicity of this proteolysis lies in the release of toxic end products of protein breakdown (proteoses) The toxicity of the proteoses has a double mechanism (*a*) Proteoses are, *per se*, toxic, causing various constitutional manifestations, and (*b*) their molecules exercise a surface action whereby the dispersion phase of lipoids is altered As a result the substrate proteins (of both primarily and secondarily involved cells) are exposed to energetic proteolysis by non-specific proteinases

The toxic protease-hydrolysis in the affected cells starts in the first stage of anaphylactic shock and goes on through the whole of the second stage, proteoses released into the blood are found there in detectable amounts soon after the injection of the shock-dose of antigen (Jobling *et al*, 1915 *c*) The whole amount of the free proteoses released into the blood throughout the period of shock is, however, not big enough to produce shock the whole blood of an anaphylactic dog, transfused into a non-sensitised dog, does not give rise to shock in the recipient (Weil, 1917) This fact has the following explanation. The main source of proteose production is the tissue involved in the anaphylactic reaction Although damaged by the anaphylactic storm, these cells are not totally destroyed and a small quantity of proteose is spilled over into the blood This relatively small quantity of proteose, however, is sufficient to inactivate the lipoids of cells not primarily affected, and to precipitate manifestations which intermingle with symptoms derived from the anaphylactic reaction The total amount of free proteose, however, does not reach the quantity required to produce peptone shock (Weil, 1917), and this fact is the reason for Weil's (1917) being unable to reproduce the anaphylactic reaction in a non-sensitised dog using as a shock-dose the whole blood of an anaphylactic dog

The toxicity of various proteoses must be discussed in the light of recent work by Menkin (1950) on inflammatory exudates. He extracted from the inflammatory exudates of serous membranes and tissues various protein derivatives, which he designated according to their pharmacological actions. Thus *pyrexin, leucotaxin, leucocytosis promoting factor, leucopenic factors, necrosin*, etc, have been isolated in pure forms and their respective toxicities have been identified in normal

animals. The toxic actions of these compounds can be traced in any form of inflammation; the anaphylactic reaction histologically represents one form of inflammation whose main distinctive feature lies in the mechanism of its initiation. Menkin rightly expressed the view that these toxins operate in anaphylactic inflammations as well. In other words, *the anaphylactic reaction, from the histological point of view, represents tissue inflammation localised in certain shock or certain ectopic organs, on this local reaction there may or may not be superimposed manifestations derived from the release of histamine and heparin, as well as from the release of the pituitary-adrenal response*

3. High titres of serum esterase have been found in the anaphylactic dog by Jobling and his co-workers (1915 *e*). The meaning of this finding has not been clearly explained. It is possible that inactivated cellular lipoids, derived from lipoprotein molecules, escape into the blood from the cells damaged by anaphylactic shock, and that the high level of lipoids in the blood, in turn, mobilises tissue esterase. Thus the increased titre of blood esterase may represent a secondary measure whereby the increased blood lipoids may be restored to their previous state.

Syndromes similar to the Anaphylactic Reaction.

The chief incentive to revision of the theory which accepts an antigen-antibody union as the basic pathogenetic mechanism of anaphylaxis was the discovery of the fact that intravenous injection of human gamma globulin into humans, or into intact or adrenalectomised non-sensitised dogs, produced manifestations similar to those of anaphylactic shock (Godlowski, 1952 *b*) In none of these conditions is the existence of specific antibodies admissible, an antigen-antibody reaction, therefore, can form the pathogenetic basis for none of these conditions By analogy, the reaction whose manifestations are identical with the above-mentioned syndromes should not be linked pathogenetically with the antigen-antibody reaction. In a review of the literature which has some bearing on the subject, conditions were found in which the presence of antibodies could certainly be excluded but whose manifestations possessed a striking resemblance to those of anaphylactic shock. These were peptone shock, *trypsin shock*, and the

reaction due to the intravenous injection of chloroform; to the same category belong reactions following intravenous injections of colloidal and highly emulsified suspensions, or parenteral administrations of heterologous sterile protein solutions, etc. In order to find a common mechanism for all these syndromes it is essential to discover the primary factors operating in each reaction, and then if possible to link them all to a single mechanism To be able to do this one must first abandon the theory of antigen-antibody reaction as the basic factor of anaphylaxis and allergy One must then discuss in detail the mechanism of each syndrome individually and find a common denominator responsible for the resemblance of syndromes of such diverse ætiology

The mechanism of **peptone shock** has already been discussed on page 30. The presence of specific antibodies is inadmissible in a dog never previously injected with peptone Yet such a dog, if injected with the proper dosage of peptone, usually develops symptoms identical with those of anaphylactic shock This fact alone should rule out the antigen-antibody reaction as the pathogenetic mechanism of both anaphylactic and peptone shock Close analysis of biochemical events operating in both types of shock will elicit the fundamental factors at work in both conditions The basic difference between anaphylactic and peptone shock is the period of sensitisation its presence in anaphylactic and its absence in peptone shock In the first stage of anaphylactic shock the toxic enzymatic system yields free intracellular molecules of proteose In its second stage these proteose molecules disrupt lipo-protein linkage, thus exposing structural proteins to non-specific proteolysis. In peptone shock, injected molecules of peptone (which chemically belongs to the proteoses) initiate chaotic, non-specific proteolysis of the substrate proteins by a similar mechanism In other words, peptone shock runs a biochemical course similar to that of anaphylactic shock from its second stage onwards. There is no sensitisation period in peptone shock and therefore no formation of a toxic enzymatic system; the role of proteose molecules has been taken over by the injected peptone molecules. The biochemical events and the symptoms produced by them are those of the second and third stage of anaphylactic shock, and hence the similarity of these two types of shock

In the reaction following intravenous injection of **human gamma globulin** the molecules of gamma globulin, deprived of their conjugation with lipoids by ether fractionation, enter various cells where they display the properties of surface-acting substances (Godlowski, 1952 *b*). Such action leads to the adsorption of cellular lipoids on their surface, thus disrupting the lipo-protein conjugation. This action, in turn, exposes the substrate proteins in the blood and in the invaded cells to an uncontrolled, non-specific proteolysis with the liberation of the various toxins described previously. Thus the reaction following intravenous injection of human gamma globulin into a man, or into an intact or adrenalectomised non-sensitised dog, starts biochemical reactions of the second and third stages of anaphylactic shock; the manifestations which follow are identical with those of anaphylactic and peptone shock.

Trypsin shock can be produced by intravenous or intraperitoneal injection of an active or inactive trypsin solution into a non-sensitised dog (Kirchheim, 1911, 1912, 1913 *a* and *b*); these observations have been confirmed by Jobling *et al* (1915 *b*). The symptomatology thus produced is identical with that of peptone and anaphylactic shock. The differentiating features between *trypsin shock* and anaphylactic shock are the absence in *trypsin shock* of a sensitisation period.

Chemically trypsin is a protein If active trypsin is administered it may display a twofold action: (1) as tryptic enzyme and (2) as protein molecules Action (1) cannot start unless protein molecules in the blood and cells are made accessible to the tryptic action by removal of the lipoid fraction from the lipo-protein molecules (*e.g*, by action (2)) Normally the blood, and all tissues, possesses enough non-specific trypsinases to proceed with hydrolysis, provided that the lipoid barrier in the lipo-protein conjugation is removed. Therefore action (1) of the injected trypsin plays an insignificant role. The main factor responsible for the production of *trypsin shock* is action (2), in which molecules of trypsin disrupt the lipo-protein conjugation by a physico-chemical mechanism. Hence the results are the same whether the trypsin is administered in its active or inactive form The disruption of the lipo-protein linkage by trypsin molecules exposes the organism's own proteins to non-specific proteolysis identical with that of the second and

third stages of anaphylactic shock · hence the identity of the manifestations of *trypsin shock* with those of anaphylactic shock.

The intravenous injection of chloroform into a dog produces manifestations which are similar to those of anaphylactic shock, but they are intermingled with symptoms derived from the direct toxic action of chloroform on the liver cells (Ratner, 1943) In this case there is a chaotic and rapid disruption of the lipo-protein linkage by the fat solvent The biochemical events which follow are similar to those of the second and third stages of anaphylactic shock, and this similarity is the cause of the resemblance between anaphylactic shock and the reaction following intravenous injection of chloroform

Non-protein colloidal solutions (*e g*, sulphur), highly emulsified non-colloidal suspensions (*e g*, fat, kaolin, Jobling *et al.*, 1915 f, iodine compounds, Pendergrass *et al*, 1942, Ramsay, 1953, and others), and heterologous protein solutions, when applied parenterally, are apt to precipitate reactions similar in their mechanism and symptomatology to those of anaphylactic shock These reactions may take place if the injected particles possess the features required for the penetration of the cell (p 21) Once in the cell, they display their disrupting action on the lipo-protein linkage and initiate the type of proteolysis found in the third stage of anaphylactic shock

In the **anti-anaphylactic state** the high titre of proteolytic and non-proteolytic antibodies released into the blood during the shock is maintained thereafter by a higher output of antibodies by the lymphoid tissue (p 33) This countermeasure prevents the penetration of the antigenic protein into the sensitised cells should another invasion of antigen occur. It operates mainly in the body fluids. Although in the anti-anaphylactic state proteases are present inside the sensitised cells, these cells have recovered from the previous shock and are as yet undamaged For this reason an escape of proteases into the blood on a large scale cannot occur (Pfeiffer, 1915).

If, however, proteases are present in the body fluids after the symptoms of acute anaphylactic shock have subsided, one may conclude (*a*) that there is an active anaphylactic focus in the body, and (*b*) that the anti-anaphylactic mechanism is

inadequate. Had it been adequate, all the antigen would have been neutralised by free antibodies and a chronic state of anaphylaxis could not have developed

To summarise. the factor common to anaphylactic shock and to the syndromes which resemble it is a rapid and uncontrolled proteolysis of the organism's own substrate proteins initiated, in the case of anaphylactic shock, by the augmented toxic enzyme system, in the cases of the anaphylactic-like syndromes by the pseudo-antigenic actions (p. 3) *This is followed by symptoms characteristic of the second and third stages of anaphylactic shock; hence the similarity between all these reactions*

The ultimate outcome of the non-specific reactions of anaphylactic type may be different in different circumstances. For example, if an organism suffering from a chronic inflammatory lesion is subjected to one of these reactions, it may even benefit from it The metabolic storm may exercise its beneficial action on inflammatory lesions by (*a*) the engagement of the pituitary-adrenal response with release of C^{11}-oxygenated corticosteroids (p. 50) and (*b*) the initiation of a proteolysis of great velocity, which causes a rapid involution of the inflammatory exudates containing highly active, but temporarily blocked, proteolytic enzymes

The Enzymatic Concept versus the Concept of Antigen-antibody Reactions in Anaphylaxis and Allergy.

The present orthodox view on the mechanism of anaphylaxis and allergy holds that an antigen-antibody reaction takes place in the sensitised cells and in the body fluids, with release of various toxins (heparin, histamine) having intrinsic and extrinsic actions. In other words, the presence of specific antibodies is essential for the initiation of the anaphylactic reaction This theory cannot explain the identity of the manifestations of anaphylactic shock with those of peptone shock, with reactions following the intravenous injection of human gamma globulin or fat solvents, or with "tryptic shock"—in all of which the presence of specific antibodies is inadmissible

The enzymatic concept could be adapted to the theory of antigen-antibody reactions as the basic phenomenon of anaphylaxis if all proteolytic enzymes were looked upon as antibodies. The formation of a specific toxic enzymatic system inside the sensitised cells could then be regarded as the formation

of a pathological antibody which by union with the specific antigen carried out a pathological proteolysis Such an approach to intracellular proteolysis cannot be accepted for the following reasons

1 Hydrolysis carried out by this enzyme liberates end products of high toxicity, whereas antibody was defined (p 4) as a substance which neutralises antigen and by such action eliminates its toxicity
2. The manifestations of anaphylactic shock are closely related to the appearance of proteoses in the body fluids and are not always related to any known antigen-antibody reaction (precipitation, agglutination, etc), furthermore, antigen-antibody interaction is very often devoid of any noxious activity in the affected organism
3 The antigen-antibody reaction is a defence measure by which the invading antigen is neutralised and immobilised, and it cannot, without alteration, be both a protection against damage and a cause of damage—or both defender and aggressor

If all proteolytic enzymes are regarded as specific or non-specific antibodies the whole process of protein catabolism should logically also be regarded as an antigen-antibody reaction Existing views on protein anabolism would then need modification, for in suitable conditions anabolism and catabolism are carried out by the same enzymes (Bergmann, 1942) Similarly, enzymatic metabolism of lipoids and carbohydrates would also have to be included in the category of antigen-antibody interaction. Thus adherence to the theory of an antigen-antibody mechanism in anaphylaxis would throw all present views on metabolism into confusion, giving in return a confused picture of anaphylaxis and allergy.

It is therefore suggested that *one should abandon altogether the view that an antigen-antibody reaction is the basic mechanism in anaphylaxis and allergy and should accept the view that two independent reactions are concerned—antigen-antibody reaction and sensitisation process*, the imperfection of the first reaction causes the second Thus the *anaphylactic reaction must be clearly distinguished from the prerequisite failure of the antigen-antibody reaction.*

The sequence of events leading to anaphylaxis and allergy may be formulated in the following way:

1 *An antigen-antibody interaction, a physiological defence measure, preserves the balance between the organism and the various antigens which normally invade every organism. This defensive mechanism, whether it operates inside or outside normal cells, neutralises the antigens and protects the organism from harm. If this defence fails, a pathological process begins.*

2. *If the invading antigen overpowers the antigen-antibody defence barrier, it induces the formation of a specific proteolytic toxic enzyme system, this process constitutes the primary mechanism of sensitisation*

3 *A later invasion of antigen* (i.e, *a shock-dose*) *initiates toxic proteolysis and liberates various proteoses; these in turn activate normal non-specific proteinases and thereby start a chaotic proteolysis of the organism's own proteins The various organic toxins which result from this proteolysis are responsible for the local and constitutional manifestations of anaphylaxis and allergy.*

CHAPTER II

HORMONAL INVOLVEMENT IN ANAPHYLAXIS

INTRODUCTION

IN the preceding paragraphs attention has been concentrated mainly on the primary proteolytic inability of cells liable to be sensitised. As a result of this inability the invading antigen molecules cause a reorganisation of the intracellular proteinases In this reorganisation a specific toxic protease is formed within the cell, and this newly formed protease splits the antigenic protein to the level of proteose during the next invasion of the sensitised cells Proteose molecules produced in small amounts inside the cell in the initial stages of the anaphylactic shock (intrinsic phase (p 33)) disrupt cytoplasmic lipo-protein molecules whereby the substrate proteins become exposed to a rapid and chaotic proteolysis exerted by the intracellular proteinases This proteolysis results in the release of various toxic intermediate protein catabolites which are responsible for various manifestations of anaphylaxis

Hormonal participation in the anaphylactic reaction may be evoked through various mechanisms In the present discussion, attention will be focused mainly on the mobilisation of the pituitary-adrenal response in acute and chronic anaphylaxis, and on the interrelation between lipoids and adrenocorticosteroids

PITUITARY-ADRENAL RESPONSE

Mechanism of Pituitary-adrenal Response.

The pituitary-adrenal response operates through five independent but closely co-ordinated mediators :

1. Peripheral chemo-receptors and neuro-receptors of excitatory or inhibitory stimuli
2. Pathways transmitting these stimuli from the peripheral receptors to the central receptors.
3. Central receptors in the hypothalamus relaying the impulses towards the adenohypophysis.

4. The adenohypophysis producing hormones which act upon the cortex of the suprarenal.
5 The suprarenal cortex producing a great variety of steroids which participate in the metabolic activities of all tissues

1 *The Chemo-receptors and Neuro-receptors* —The stimuli acting on the peripheral tissues from the external or internal environment may be of physical, chemical, or emotional character (p. 78) They are recorded by chemo-receptors and neuro-receptors, and are eventually transmitted to the autonomic nervous centres

2 *The Pathways Transmitting Recorded Stimuli* —The pathways transmitting stimuli from the periphery are neural and humoral in nature Stimulation of the autonomic nervous system may affect directly the relaying centres in the hypothalamus (Vogt, 1951) High cervical transection of the spinal cord, however, does not prevent the mobilisation of the pituitary-adrenal response, in these circumstances this response is mediated by a humoral pathway, *i e*, by adrenaline liberated from the medulla of the adrenal glands (Long, 1947), functioning through its intact secretory nerve supply (Harris, 1948) Physiologically, the central receptors may be stimulated simultaneously through both these pathways

3 *Hypothalamus as Relaying Organ.*—The neuro-humoral stimuli brought to the hypothalamus are relayed in the region of the median eminence, this results in " . . . the liberation of a chemo-transmitter which is transported by the portal circulation to excite or inhibit secretory action of the adenohypophysis " (Quotation from Harris, 1951, reported previously by Harris, 1944, and Green *et al*, 1947.)

4. *Secretory Action of Adenohypophysis* —There is no evidence that the adenohypophysis (in humans) possesses neuro-secretory fibres (Harris, 1951). In rabbits, Vaques-Lopez (1949) had demonstrated neural fibres connecting the hypothalamic area with the adenohypophysis It is at present believed that anterior pituitary secretion can be stimulated by three mechanisms which may operate simultaneously or independently These are .

(*a*) Release of adrenaline by the sympathetico-adrenal system.

(b) Fluctuations of the concentration of adreno-cortical hormones in the body fluids

(c) Direct hypothalamic stimulation

(a) *The adrenaline and adrenaline-like compounds* released from the adrenal medulla and from other chromaffin tissues *may influence the secretory activity of the adenohypophysis* (i) via the hypothalamus (Gershberg *et al*, 1950), or (ii) by directly stimulating the cells of the adenohypophysis to secrete adrenocorticotropic hormone (ACTH) (McDermott *et al.*, 1950), or (iii) by promoting the excessive utilisation of corticosteroids by the peripheral tissues, thus lowering the steroid level in the blood, which in turn stimulates the release of ACTH (Sayers, 1950 *b*).

(b) *The fluctuation of the concentration of corticosteroids in the body fluids* has been experimentally demonstrated to be an inhibitory or excitatory stimulus for anterior pituitary action The repeated administration of cortisone caused depression of ACTH secretion (Sayers and Sayers, 1947), whilst repeated stress stimuli (which caused increased utilisation of the corticosteroids) exercised a stimulating effect on the secretion of ACTH (Sayers and Sayers, 1948)

(c) *Direct stimulation of the hypothalamus* in the region of the tuber cinereum or mammillary bodies produces circulating lymphopenia (de Groot *et al*, 1950, Hume *et al*, 1950) This is considered to be the result of the release of lympholytic corticosteroids through ACTH action (Dougherty *et al*, 1946, *a* and *b*, Whyte *et al*, 1946)

5 *Response of the Suprarenal Cortex to ACTH*—The exact mechanism of ACTH action on the suprarenal cortex is unknown. It is known that the administration of ACTH stimulates all known functions of the adrenal cortex, and this is evidenced by (a) hypertrophy and hyperplasia of the cortex (reviewed by Sprague, 1951), (b) decreased content of ascorbic acid (Sayers *et al*, 1945), and cholesterol (Long, 1947) in the cortex of the adrenals, (c) increased urinary output of cortical steroids (critically reviewed by Sayers, 1950 *b*), (d) atrophy of lymphoid tissue and thymus (Selye, 1946), and fluctuations in circulating eosinophils and lymphocytes (Thorn *et al*, 1948). The practical value of the "eosinopenic test" (Thorn *et al*, 1948) in assessing adrenocortical activity seems somewhat

diminished by new light thrown on the mechanism of hormonal eosinopenia and lymphopenia.

Relation of Pituitary-adrenal Response to Anaphylaxis.

The stimulus of a chronic or acute anaphylactic reaction evokes the pituitary-adrenal response, and the release of cortical steroids precipitates symptoms which are superimposed existing manifestations derived, directly or indirectly, from the anaphylactic reaction

There is strong suggestion that C^{11}-oxygenated corticosteroids exert a powerful influence on the catabolic phase of intracellular protein metabolism (Engel, 1949 and 1951; Hoberman, 1950) This hypothesis was corroborated, both *in vitro* and *in vivo*, in experiments in which these steroids exhibited their destructive action on white blood cells (Godlowski, 1952, Muehrke *et al*, 1952) The histological evidence of this action is a rapidly progressive involution presenting in the nucleus as pyknosis, karyolysis, and karyorrhexis, and in the cytoplasm as vacuolisation, degranulation, and loss of staining affinity In its extreme forms, such destructive action causes complete disintegration of the cell, leaving in its place an amorphous mass including the débris of the nucleus and granules (p 90) Since this leucolytic action of corticosteroids occurs also *in vitro* (Godlowski, 1952 *a*; Muehrke *et al.*, 1952), *it is reasonable to postulate that under these experimental* conditions corticosteroids exert a direct action on all white blood cells. Dougherty *et al* (1944, *a* and *b*) and White *et al.* (1946) showed that ACTH injected into intact animals causes a gross involution of lymphoid tissue by lympholysis, and that such an effect cannot be obtained in adrenalectomised animals. From all these experimental data it is reasonable to assume that *ACTH enhances the catabolism of protein not only in lymphocytes* but in all white blood cells, by stimulating the release of leucolytic cortical steroids It has been conclusively proved that in association with the cytolytic action of corticosteroids the NPN in the blood rises significantly (Pearson *et al.*, 1949, Engel, 1949, 1951, *a* and *b*). These findings may be interpreted as showing that the intracellular proteolysis enhanced by corticosteroids splits proteins to the level of NPN. It is known, on the other hand, that such an action is carried

out by proteinases of the tryptic type (Holman *et al*, 1947, and others) Therefore one can further assume that corticosteroid cytolytic action may result from the direct or indirect stimulation of the intracellular catabolic phase of the metabolism of protein

INTERRELATION BETWEEN LIPOIDS AND CORTICOSTEROIDS

Influence of Exogenous Corticosteroids on Blood Lipoids.

In connection with the mediation of corticosteroids in the proteolysis whose results have been observed in white blood cells it may be of some value to discuss the interrelation between lipoids and corticosteroids in the light of present knowledge in this field

There are contradictory reports on the response of lipoids to the administration of leucolytic suprarenal hormones In a short experiment in normal humans Conn and his associates (1949) showed that injection of ACTH (30 to 100 mg) caused a marked decrease in total serum cholesterol, due chiefly to the reduction in the ester fraction Their explanation was that in this experiment cholesterol served as a material from which cortical steroids were synthesised Bloom *et al* (1952) found that ACTH and cortisone given in dosage sufficient to ameliorate various allergic conditions did not significantly affect serum cholesterol, they found, however, that in normal rabbits treated with ACTH severe lipæmia and cholesterinæmia resulted Further experiments on animals (Kobernik *et al*, 1950, Katzin *et al.*, 1950, Rich *et al*, 1951) and prolonged observations on humans (Aldersberg *et al.*, 1950, Wolfson *et al*, 1950) have demonstrated a definite increase in serum phospholipoids and in both fractions of cholesterol after the administration of ACTH and cortisone

The discrepancy in the reports on fluctuation of blood lipoids in various species of animal and in men following the administration of cortisone and its allied substances has its source probably in the technique used in these experiments. The main site of chemical activity of corticosteroids is in the cells, therefore lipoid fluctuations in the blood may or may

not reflect the actual cyto-chemical alterations. Hence one may expect to obtain more information on this problem from the quantitative and qualitative histo-chemical analysis of the tissue lipoids after the administration of corticosteroids.

In the following paragraph an attempt has been made to adduce evidence that C^{11}-oxygenated corticosteroids participate in protein metabolism by saturation of the unsaturated carbon atoms in the fatty acids of the lipoids in lipo-protein molecules In such a concept the elevation of the blood lipoids which accompanies prolonged administration of these corticosteroids *may be referred to the mobilisation of intracellular lipoids by* the corticosteroids themselves , by this action corticosteroids disrupt the lipo-protein linkage of the cytoplasmic molecules (by chemical mechanism, p 108), thus initiating protein hydrolysis, and liberated lipoids escape from the cells into the blood This contention derives valuable support from the co-existing slight elevation of the proteolytic power of the serum (Holman *et al* , 1947 , Sayers *et al* , 1949 , Godlowski, unpublished data)

Mechanism of Lipoid Inactivation by Corticosteroids.

Before entering a discussion on the theoretical and practical applications of the above-mentioned hypothesis it would be advisable to discuss the facts which led to the suggestion of the mechanism of elevation of the blood lipoids during prolonged administration of the C^{11}-oxygenated corticosteroids

1 It is well established that if normal blood serum is treated with fat solvents such as chloroform, ether, acetone, etc , tryptic hydrolysis of its own proteins begins, both *in vitro* and *in vivo* , this proteolysis is caused by the removal of the lipoids (Fig. 3) which protect the blood proteins against tryptic enzymes normally present in the blood (Schwartz, 1909 , Sugimoto, 1913 , Jobling *et al.*, 1914)

2 It has been proved that the antitryptic action of lipoids is directly connected with the presence of unsaturated carbon atoms *in the fatty acids of blood lipoids* , *any oxidising agent* (such as hydrogen peroxide or iodides, etc.) can destroy this antitryptic action of lipoids by breaking the double bonds of the unsaturated carbon atoms in the fatty acids (Jobling *et al* , 1914 and 1915 *a*).

3. Desoxycholic acid, which structurally is closely related to the corticosteroids (reviewed by Jacobsen *et al*, 1951), under suitable experimental conditions (Wieland *et al.*, 1916) can form "a co-ordination compound" with fatty acids. This newly formed compound of eight molecules of desoxycholic acid and one molecule of fatty acid acquires new physico-

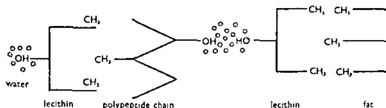


FIG 3

Relation between polypeptide side-chains and lecithin, O = water molecules (From A Frey-Wyssling, "Submicroscopic Morphology of Protoplasm," Second English Edition, 1953 Elsevier Publishing Co Inc, New York and Amsterdam)

chemical features such as altered solubility, changed melting point, etc

If one may be allowed to speculate in human biochemistry the following mechanism of the chemical inactivation of lipoids by the corticosteroids might be accepted The oxidation potential of cortisone and its allied steroids (Anderson *et al*, 1951) lies in the oxygen atoms or hydroxyl groups attached to C^3, C^{11}, and C^{17} It has been proved (Kendall, 1949, and others) that C^{11} and C^{17} possess a key position guaranteeing the known biological effects of these steroids (effecting protein metabolism) Therefore one can further assume that the oxygen attached to the C^{11} and C^{17} is the element which breaks the double bonds of the fatty acids, thus inactivating cellular and blood lipoids

The reduction potential of corticosteroids lies in the reducing group attached to the C^{17}, saturation of the double bonds in the fatty acids by hydrogenation can safely be excluded for two reasons (1) In the living cell oxidation is more easily carried out than reduction of the fatty acids, and it has been *shown (Jobling et al., 1914) that oxidation is the process which* destroys the insulating action of lipoids; (2) corticosteroids in which the reducing group is attached to C^{17} and which possess no oxygen in the positions C^{11} and C^{17} (*e g.*, desoxycorticosterone),

do not have the biological action connected with increased proteolysis (critically reviewed by Fieser, 1950)

The quantity of C^{11}-oxygenated corticosteroids required for the inactivation of one molecule of fatty acid depends on the number of unsaturated carbon atoms, which varies in different fatty acids Therefore the degree of protein catabolism would vary not only with the quantity of hormone given but also with the chemical composition of the lipoids which insulate the protein

Effects of Lecithin on Biological Action of Corticosteroids.

These theoretical speculations have been tested in an experiment in which *ovo-lecithin* was used as phospholipoid. The rationale of this experiment was as follows. if C^{11}-*oxygenated corticosteroids inhibit the protecting action of* lipoids against tryptic proteolysis by saturation of fatty acids, then, vice versa, excessive amounts of lipoids (ovo-lecithin) should exhaust the oxidation potential of C^{11}-oxygenated corticosteroids, thus blocking their biological activity.

In one group of experiments cortisone acetate (15 mg.), incubated for one hour at 37° C, was tested for its eosinolytic and lympholytic potency on adrenalectomised dogs. A few days later the same dogs were injected with 15 mg. cortisone acetate which had previously been incubated at 37° C. for one hour with 150 mg of ovo-lecithin suspended in distilled water (5 per cent) The eosinolytic and lympholytic potency of cortisone was completely eliminated

In another group of experiments, adrenalectomised dogs were intravenously injected with 20 ml. of a 5 per cent highly emulsified ovo-lecithin suspension in distilled water, fifteen minutes afterwards 15 mg of cortisone acetate was injected subcutaneously During the next four hours the dogs were injected intravenously every half-hour with 3 ml of the same suspension of ovo-lecithin The eosinolytic and lympholytic action of cortisone acetate was grossly reduced

The data so far obtained in the experiments in which the blocking action of lecithin on the biological activity of cortisone has been assessed support the theoretical speculations The number of the experiments, however, is too small and their nature too limited to draw any definite conclusions

Physico-chemical, Physical, and Chemical Factors Inactivating Lipoids.

Cellular and blood lipoids possess the capacity of insulating the substrate proteins against the tryptic action of proteolytic enzymes in the manner shown in Fig 3 The *destruction* of this insulating barrier may be achieved either by the disruption of the lipo-protein conjugation by physical and physico-chemical factors, or chemically by saturation of the unsaturated carbon atoms (Jobling *et al.*, 1915*c*), the exact role played by the unsaturated carbon atoms in the insulating action of lipoids is not known

The factor which inactivates the lipoids' insulation of substrate proteins against proteolytic action may operate through at least three mechanisms

1. *Physico-chemically*, by the alteration of the dispersion phase of the lipoids in the lipo-protein molecules, colloidal substances whose particles possess high surface-acting forces disrupt the lipo-protein conjugation by adsorption of the lipoid molecules on the large surface of their molecules. In this way protein or proteose molecules which have escaped proteolysis and have penetrated various cells (*e g*, in human gamma globulin reaction or in peptone shock) or have been produced inside the cell (*e g*, in anaphylactic shock) *disrupt the lipo-protein equilibrium*, thereby exposing substrate protein to proteolytic action.
2. *Physically*, by intravenous injection of fat solvents which remove lipoid molecules from the lipo-protein molecules, this action leads to the same disturbance of lipo-protein equilibrium as described in (1).
3. *Chemically*, by saturation of double bonds of the unsaturated carbon atoms in the fatty acids by various methods such as oxidation, hydrogenation, or halogenation (Deuel, 1951), whereupon the lipoids are rendered incapable of protecting the substrate proteins

The first and the third methods of inactivation of lipoids take place in different stages of anaphylactic shock: the

physico-chemical method operates in the second stage of anaphylactic shock and the chemical method, which operates in the third stage, is carried out by corticosteroids released through the pituitary-adrenal response; the latter type of the initiation of proteolysis continues proteolysis of a physiological pattern at a rapid rate which is characteristic for the third stage of anaphylactic shock

Each of the above mechanisms results in the disruption of the lipo-protein conjugation in a different manner and with different consequences The fundamental result of all these mechanisms is, however, the same: the breakdown of the organism's own substrate proteins and the dissociated lipoids from the lipo-protein conjugation may leave thereafter the interior of the cells. This may explain the elevated lipoid concentration in the body fluids found after the administration of C^{11}-oxygenated corticosteroids (Kobernik *et al.*, 1950; Katzin *et al*, 1950, Adlersberg *et al*, 1950, Wolfson *et al.*, 1950, Rich *et al*, 1951 Bloom *et al*, 1952) The flooding of the blood with lipoids may, in turn, induce the liberation of tissue esterase to dispose of the excess blood lipoids This may be the cause of the high level of lipoids and of esterase in the blood which is found in anaphylactic shock.

The inactivation of blood and cellular lipoids, leading to the initiation of proteolysis, is a process the velocity of which gradually increases This action of corticosteroids may be regarded as a physiological mechanism for the initiation of protein catabolism, whereas the physico-chemical (by surface-acting forces) and physical (by fat solvents) inactivation of lipoids may be looked upon as examples of pathological initiation of proteolysis. The rapid and disorderly proteolysis initiated by the last two factors leaves behind partially catabolised protein breakdown products of high biological toxicity (proteoses) and liberates physiological substances which, because of the rapidity of their release, acquire a pathological potency (histamine and heparin) The chemical method of initiation of proteolysis (by the release of cortico-steroids) may be considered in these circumstances as a counter-measure whereby the toxic catabolites are disposed of by normal proteolysis.

Tentative Explanation of the Action of C^{11}-oxygenated Corticosteroids in Anaphylaxis.

It has been conclusively proved in animal experiments that cortisone and its allied substances have no effect on the course of anaphylactic shock itself (Leger *et al*, 1948, Dworetzky *et al*, 1950) The improvement in allergic conditions obtained by the administration of these corticosteroids must, therefore, operate through a mechanism other than a direct effect on the anaphylactic shock which is a basic pathogenetic factor in allergy. It is suggested that the mechanism of the therapeutic action of corticosteroids in allergy is through chemical inactivation of the lipoids in the lipo-protein whereby physiological proteolysis is initiated The velocity of this proteolysis will depend primarily on (1) the kinetics of the proteolytic enzymes available in the tissues involved and (2) the rate of the disruption of the lipo-protein molecules by the corticosteroids

The cellular and extracellular anaphylactic inflammatory exudates contain large quantities of highly active proteolytic enzymes (Weiss *et al*, 1935, Menkin, 1950, and others) If the C^{11}-oxygenated corticosteroids administered reach the quantity required for the chemical inactivation of lipoids in lipo-protein molecules, a rapid proteolysis begins The accelerated physiological proteolysis which follows eliminates exudates accumulated in the anaphylactically inflamed tissues Thus C^{11}-oxygenated corticosteroids exert their **beneficial effects through the elimination of the secondary histopathological reaction due to the biochemical activities of the third stage of anaphylactic shock (p. 34), leaving the primary pathogenic mechanism of toxic proteolysis of the first and second stages of the anaphylactic shock unaffected.**

In other words, C^{11}-oxygenated corticosteroids initiate physiological proteolysis by a systematic and co-ordinated inactivation of lipoids in lipo-protein molecules, according to the proteolytic capacity of the available proteolytic enzymes. Hence the therapeutic potency of cortical hormones depends on the kinetics of the proteolytic enzymes available at the time of the administration of the cortical hormones Granulation tissue, cellular and fluid components of the inflammator exudates, all possess a high content of very active proteinase

and therefore all these elements are very susceptible to the proteolysis initiated by cortical hormones. Lymphoid tissue containing as it does a large amount of highly active proteolytic enzyme (Drinker *et al.*, 1941; Fruton, 1946; and others), is also highly susceptible to hormonal proteolysis, which leads to involution (Selye, 1946). Eosinophils, cells which have recently reorganised their intracellular enzymatic system in the process of eosinophilisation (Godlowski, 1952), possess highly active proteolytic enzymes, and are also highly susceptible to hormonal proteolysis Neutrophil leucocytes containing highly kinetic proteinases (Weiss *et al*, 1935, Drinker *et al*, 1941, Rich, 1944) respond equally well to the proteolysis induced by steroids, their destruction, however, is not reflected in the blood, because the co-existing hyper-mobilisation of neutrophil leucocytes from the bone-marrow masks the rapid leucolysis Polymorphonuclear leucolysis, however, has been clearly demonstrated *in vitro* and in experiments *in vivo* in which a perfused organ was separated from the general circulation and the perfusing blood was subjected to the cytolytic action of corticosteroids (for details see pp 88-91).

The ability of C^{11}-oxygenated corticosteroids to induce proteolysis in a tissue containing proteolytic enzymes may lead to the dissemination of a quiescent infection, *e g*, tuberculosis (Michael *et al*, 1950, Hart *et al.*, 1950, and others). A focus of infection in its inactive stage is cut off from direct circulatory contact with the rest of the organism by a wall of tissue which consists mainly of epitheloid cells, lymphocytes, polymorphonuclear leucocytes, and other cells characteristic of young granulation tissue, all these cellular elements are abundantly provided with proteolytic enzymes (Weiss *et al*, 1935, Rich, 1944) Hence rapid hormonal *proteolysis induced by corticosteroids destroys* the barrier surrounding the focus, thereby facilitating the hæmatogenous spread of infection

Green, on the ground of experimental work on mitosis done in association with his co-workers (Green, 1950, Green *et al*, 1951, *a* and *b*), came to the conclusion that cortisone inhibits the anabolic activity of cells. Short-lived mesenchymal elements which manufacture various antibodies die out in the course of the normal ageing process, and they are not replaced because of the inhibition of the anabolic phase by corticosteroids.

Our knowledge about protein anabolism is very scanty There is, however, good reason to believe that the same proteolytic enzymes are engaged in both anabolic and catabolic action (Bergmann, 1942) Reversion of metabolism from one action to another depends on the contemporary requirement of the chemical environment in which the metabolic activity takes place. Therefore the inhibition of mitosis seen by Green and his co-workers in tissue cultures *in vitro* and *in vivo* may result from both actions; proteolytic enzyme engaged in catabolic activity is automatically excluded from the anabolic action The final morphological results are therefore due to two co-existing components aplastic (due to the inhibited anabolism) and destructive (due to the stimulated catabolism)

CHAPTER III

THE ROLE OF EOSINOPHILS IN ANAPHYLAXIS

INTRODUCTION

Hæmatopoietic and Non-hæmatopoietic Formation of Eosinophils.

THE *origin and function of eosinophils are as yet not conclusively established.* *There are two opposing theories* of eosinophil formation

1. *Leucoblastic* in the bone-marrow (Ehrlich, 1878-79),
2. *In non-hæmatopoietic tissues.*

The protagonists of the first theory maintain that the presence in the bone marrow of eosinophilic myelocytes as well as all other forms of maturation of eosinophil, including those seen in the circulating blood, strongly supports the view that eosinophils are formed in the bone-marrow In other words, the formation and maturation of eosinophils takes place in the bone-marrow, and the mature forms of eosinophils are discharged into the circulation by the same mechanism as that by which the rest of the white cells formed in the bone-marrow are released into the blood

The non-hæmatopoietic formation of eosinophils is less widely accepted, none the less, arguments adduced by its supporters possess also a sound basis. Many cytologists (Dominici, 1909, Ringeon, 1921, Gauthier-Villars, 1950; and others) have observed eosinopoiesis similar to that seen in the bone-marrow in various non-hæmatopoietic tissues in experimental animals, without an increased eosinopoiesis in the bone-marrow. In other words, in some organs (intestine) of these animals, a constant eosinopoiesis takes place physiologically, pathologically, *e g*, in some inflammations, early and mature forms of eosinophils *are seen in the vicinity of inflamed* foci (Maximow, 1927, Gauthier-Villars, 1950) If the presence of early, transitional, and mature forms of eosinophils is a valid argument for the medullary theory, it should also hold good for the formation of eosinophils in non-hæmatopoietic tissues.

The truth, therefore, seems to be that eosinophils are formed in the bone-marrow as well as in non-hæmatopoietic tissues, wherever the conditions for their formation may exist Therefore the elucidation of the mechanism of eosinophil formation is more important than the dispute about the place of their origin

MECHANISM OF EOSINOPHIL FORMATION

Factors Conditioning Eosinophil Formation.

The main principles of eosinophil formation may be summarised as follows

1. Any cell in the living organism invaded by molecules of antigenic protein may become eosinophilic, provided
2. The enzymatic structure of the cell which is about to be eosinophilised bears features of an insufficiency in intracellular proteolysis Such a cell bears, therefore, the features of an inadequacy as regards the performance of normal functions
3. An unsplit protein molecule possessing antigenic capacity invading such a cell exerts a stimulus for the reorganisation of the intracellular proteolytic enzymes This reorganised enzyme system is able not only to catabolise the invading molecules but also, in the anabolic phase, to reconstitute the antigenic protein and to incorporate it into the structural proteins of the cell (for details see p 73)

Classification of Eosinophils.

From the point of view of the existing embryological classification, such a concept is inadmissible because all morphological elements of the blood have been proved to be of mesodermal origin In view of the evidence of the formation of eosinophils in tissues of non-mesodermal origin (*e g*, epithelial lining of the intestinal crypts), a formula has to be adopted which admits the existence of two types of cells with eosinophilic granulations in the blood.

1. *True blood eosinophils* formed in the bone-marrow or in other hæmatopoietic organs.

2. *Pseudo-eosinophils* formed physiologically or pathologically *in various non-hæmatopoietic organs*, and subsequently mobilised and shifted into the blood.

Genetically, the latter type of eosinophil does not belong to the blood elements The "true blood eosinophils" are descendants of immature white blood cells (promyelocytes or still earlier forms) which became eosinophilised in the bone-marrow by the same mechanism as that of the eosinophilised *cryptal epithelial cells.* The protein molecules which invade the immature leucoblastic cell may be of endogenous origin, *e g.*, hæmoglobin, which was found normally to be free in the bone-marrow (Ringeon, 1938) The protein which initiates eosinophilic transformation of the leucoblastic cells in the bone-marrow may well be also of exogenous origin, *e g*, protein absorbed in the alimentary tract and disseminated all over the organism by the hæmatogenous spread. This physiopathological alteration, viz., eosinophilic transformation, deprives the eosinophylised cell of its original morphology and primary function

Effects of Antigenic and De-antigenised Diets on Circulating and Tissue Eosinophils.

The tissue eosinophils in the lamina propria of the intestinal mucosa in dogs have been seen by many workers (Kultchitzky, 1897, Simon, 1905, Biggart, 1932, and others), and all of them agree that the number of eosinophils is somewhat related to the ingestion of proteins The early forms of eosinophils which were also found there were described as eosinophilic *myelocytes (Simon, 1905) In an attempt to explain* this phenomenon it is advisable first to review briefly the literature which has some bearing on this subject.

Absorption of unsplit protein from the intestinal tract takes place physiologically, but only in quantities which are insignificant so far as the nutritive value of proteins is concerned. Alstyne *et al* (1911), by using a very sensitive precipitin test, have clearly established the fact that unsplit protein is absorbed from the alimentary tract of the guinea-pig Sussman *et al.* (1928) and Brunner *et al* *(1928) confirmed these experiments*

on human material in various anaphylactic conditions In intestinal disorders associated with impaired digestion, the absorption of unsplit proteins is greatly increased, the ingestion of various antigens may give rise to the symptoms of anaphylaxis (Verzàr *et al*, 1936) From the experimental works of Haidenhain (1888), Opie (1904), Simon (1905), Biggart (1932), and others, done on guinea-pigs, rabbits, and dogs, there appears to be a close correlation between the tissue eosinophils of the intestinal mucosa and the ingestion of proteins.

Various forms of eosinophilised cryptal cells are described in the literature under various terms, *e g*, *Paneth cells* or *Globular leucocytes* (quoted by Moximow and Bloom, 1948), the origin and function of which are not clearly defined *Kultchitzky cells* (Kultchitzky, 1897) are those elements in the intestinal crypts which are nearest in their histology and suggested function to the " transitional eosinophils " described elsewhere in this essay. Duran-Jorda (1943) suggested that eosinophils may be formed in the mucosa of the alimentary canal from lymphocytes passing through the stage of Paneth cells.

In an attempt to elucidate the connection between intestinal eosinophilia and the ingestion of proteins, two separate experiments were carried out for the reinvestigation of this problem: feeding animals with a highly antigenic diet, and alternatively with a de-antigenised diet

Tables 2A, 2B, and Fig. 4 show the data of the experiment in which two normal dogs were kept for five days on a raw-meat diet. On the sixth day both were laparotomised and biopsies taken from the stomach, duodenum, jejunum, ileum, and colon, biopsies from the bone-marrow (rib) and from a mesenteric lymph node were taken also at the same time For the next five days (post-operative catabolic phase) the dogs were kept on a light mixed diet In the succeeding ten days both animals were put on amino acids diet, described by Silber and his associates, which consists of ten essential amino acids and covers the N requirement (Silber *et al*, 1949) This diet was supplemented with a vitamin mixture, salts, lard, dextrin, and water, and it supplied 80 calories per kilo, according to the recommendations of Allison *et al.* (1945). On the eleventh day of this diet the dogs were laparotomised again, and biopsies

taken from the same areas. In the next seven days the dogs were kept on normal diet and, at the end of this period, they were killed and specimens of all organs taken for histological

TABLE 2A

Changes of Protein Content in Diet reflected in Circulatory Eosinophilia

Dog no	Day to day circulating eosinophils/cu mm during 5 days raw meat diet	Day-to day circulating eosinophils/cu mm during 5 days light diet of post-operative period	Day to-day or alternate days circulating eosinophils/cu mm during 10 days amino acid diet	Day to day or alternate days circulating eosinophils/cu mm during 7 days mixed diet
1	504	230	870	63
	664	415	517	67
	730	620	299	119
	810	885	99	196
	900	1075	144	328
	—	—	265	—
	—	—	30	—
2	560	0	715	163
	642	93	1040	399
	805	158	604	359
	850	214	275	460
	978	520	390	538
	—	—	133	—
	—	—	100	—

TABLE 2B

Changes of Protein Content in Diet reflected in Tissue Eosinophilia of Alimentary Tract

	Tissue eosinophil count/H P F after 5 days raw meat diet (mean ± S D)			Tissue eosinophil count/H P F after 10 days amino acids diet (mean ± S D)			Tissue eosinophil count/H P F after 7 days mixed diet (mean ± S D)		
		Eosinophils			Eosinophils			Eosinophils	
Dog no	Organ	Transitional	Mature	Organ	Transitional	Mature	Organ	Transitional	Mature
1	Stomach	0	1 ± 0.10	Stomach	0	1 ± 0.06	Stomach	0	2 ± 0.15
	Duodenum	24 ± 0.50	38 ± 0.40	Duodenum	3 ± 0.21	12 ± 0.60	Duodenum	8 ± 0.20	33 ± 0.20
	Jejunum	14 ± 0.20	48 ± 0.61	Jejunum	3 ± 0.19	14 ± 0.11	Jejunum	6 ± 0.11	38 ± 0.5
	Ileum	18 ± 0.41	75 ± 0.52	Ileum	4 ± 0.19	20 ± 0.46	Ileum	6 ± 0.72	49 ± 0.6
	Colon	8 ± 0.10	5 ± 0.15	Colon	1 ± 0.08	2 ± 0.15	Colon	2 ± 0.1	6 ± 0.42
2	Stomach	0	4 ± 0.2	Stomach	0	0.5 ± 0.10	Stomach	0	0
	Duodenum	20 ± 0.1	50 ± 0.62	Duodenum	8 ± 0.03	14 ± 0.22	Duodenum	10 ± 0.31	38 ± 0.4
	Jejunum	24 ± 0.22	52 ± 0.35	Jejunum	3 ± 0.10	18 ± 0.36	Jejunum	15 ± 0.48	33 ± 0.25
	Ileum	25 ± 0.51	65 ± 0.44	Ileum	5 ± 0.04	28 ± 0.44	Ileum	11 ± 0.53	45 ± 0.72
	Colon	8 ± 0.06	25 ± 0.90	Colon	2 ± 0.12	3 ± 0.40	Colon	3 ± 0.10	9 ± 0.36

The mean values are of 30 H P F readings.

analysis All these periods of various diets were preceded, accompanied, and followed by the "circulating eosinophil count" (p. 79).

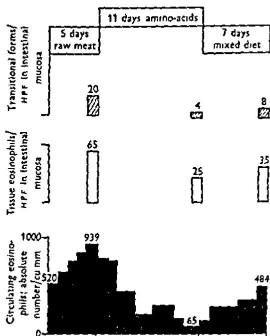


Fig. 4

The effect of dietary changes on eosinophilia

From the data shown in Tables 2A and 2B and diagrammatically presented in Fig. 4 the following conclusions can be drawn:

1 In a normal dog a raw-meat diet creates conditions in which the formation of transitional eosinophils in the intestinal crypts is greatly increased; during this stage a very high blood eosinophilia and high intestinal eosinophilia develops

2. A subsequent complete elimination of proteins from the diet, and their replacement by amino acids, reduced the eosinophilisation of the cryptal epithelium. Ten days of such a diet, however, cannot completely suppress eosinophilisation

3. Circulating eosinopenia during the period of protein-free diet may be regarded as resulting from the suppression of eosinophil formation in the cryptal epithelium.

Effects of the Diet with De-antigenised Proteins on Intestinal Eosinophilia.

In an attempt to elucidate the influence of the nature of the ingested proteins on the eosinophilisation of the cryptal epithelium an experiment was carried out on mice. Mice were chosen for this experiment because, when kept on ordinary diet (rat-cubes manufactured by the Agricultural Co-operative Society Ltd, Aberdeen), they do not possess eosinophils in any form in the mucosa of the intestine, nor do they develop an intestinal lymphocytic barrier to any great extent, they do possess, however, at the bottom of the intestinal crypts, numerous zymogen secreting cells (Paneth cells). These cells in mice differ markedly in their histological appearance from the transitional eosinophils (if these forms of eosinophilised cells appear in the mucosa) The rat-cubes are prepared from various cereals, salts, vitamins, fish, meat, and dried milk. In the course of preparation these ingredients are subjected to a temperature of 100° C or more, and to a pressure of 25 lb per sq in or greater, both for over one hour. By these procedures the proteins of the rat-cubes became denatured, and they lost their antigenicity (Ratner *et al*, 1935) but retained their nutritive value

In this experiment thirty mice of both sexes, of Swiss strain, kept on the above diet, and thirty mice on denatured milk both served as control The experimental series consisted of four groups of ten mice each, and all of them were fed on fresh milk only, for various periods as shown in Tables 3A and 3B At the end of each period, the whole group was killed The intestinal tract, spleen, and bone-marrow of each animal were then histologically analysed.

The results of these experiments were as follows:

1 Normal white mice kept on a de-antigenised diet do not develop a lymphocytic barrier to any great extent in the lamina propria of the absorptive part of the alimentary canal. Eosinophils are completely absent from their intestinal mucosa, numerous Paneth cells are at the bottom of the intestinal crypts closely grouped one beside the other

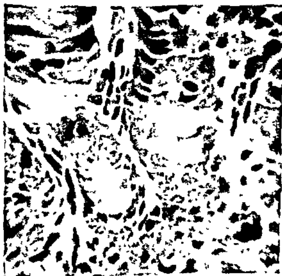
2. A moderate number of early and mature eosinophils is present in the subcapsular and trabecular areas of the spleen, and also in the bone-marrow.

Fig 5



Jejunum of a mouse for thirty days on fresh milk diet In the bottom of the crypt numerous columnar cells (Paneth cells) filled with large eosinophilic granules formed by zymogenic material partly discharged into the lumen of the crypt Staining, azo eosin Magnification × 800

Fig 6



Jejunum of the same mouse as in Fig 5 with numerous transitional eosinophilic epithelial cells with no resemblance to Paneth cells. Numerous mature eosinophils and lymphocytes in lamina propria Staining, azo eosin Magnification × 800.

[*To face page* 66

TABLE 3A

Effects of Denatured (De-antigenised) Proteins in the Diet on Circulating Eosinophils.

Blood eosinophils of 30 mice: permanent diet of denatured protein (mean ± S D)	Blood eosinophils of 30 mice 30 days on denatured milk (mean ± S D)	Blood eosinophils of 10 mice on fresh milk for 10 days (mean ± S D)	Blood eosinophils of 10 mice on fresh milk for 20 days (mean ± S D)	Blood eosinophils of 10 mice on fresh milk for 30 days (mean ± S D)	Blood eosinophils of 10 mice on fresh milk for 60 days (mean ± S D)
160 ± 5	172 ± 6	192 ± 5	189 ± 10	662 ± 15	210 ± 11

TABLE 3B

Effects of Denatured (De-antigenised) Proteins in the Diet on Tissue Eosinophils.

		Tissue eosinophils of 30 mice: permanent denatured protein (mean ± S D)	Tissue eosinophils of 30 mice 30 days on denatured milk (mean ± S D)	Tissue eosinophils of 10 mice on fresh milk for 10 days (mean ± S D)	Tissue eosinophils of 10 mice on fresh milk for 20 days (mean ± S D)	Tissue eosinophils of 10 mice on fresh milk for 30 days (mean ± S D)	Tissue eosinophils of 10 mice on fresh milk for 60 days (mean ± S D)
Bone marrow	Transitional	2 ± 0·10	1·8 ± 0·10	2 ± 0·21	2 ± 0·10	1·6 ± 0·28	1 ± 0·05
	Mature	4 ± 0·50	5 ± 0·08	3 ± 0·18	3 ± 0·26	3 ± 0·18	20 ± 0·15
Spleen	Transitional	+	+	+	0	0	0
	Mature	+ +	+ +	+ +	+ +	+ +	+ +
Small intestine	Transitional	0	0	0	0	2 ± 0·25	2 ± 0·31
	Mature	0	0	5 ± 0·35	7 ± 0·20	16 ± 0·60	10 ± 0·17
	Paneth cells	+ +	+ +	+ +	+ +	+ +	+ +

The mean values are taken from 90 h p f readings of duodenum, jejunum, ileum 30 h p f of each

+ + few cells in each h p f   + 0 to 1 cell every few h p f

Denatured milk boiled for 1 hr under 25 lb pressure

3. A great number of Paneth cells is present in the crypts in the control and in the experimental series; they are histologically different elements from the transitional eosinophils of the cryptal epithelium (Figs. 5 and 6).
4. After the first ten days of the fresh milk diet, mature eosinophils begin to appear in the villi of the small intestine, thereafter their number gradually increases.
5. Between the twentieth and thirtieth days of fresh milk feeding, transitional forms of eosinophils begin to *appear in the lower parts of the intestinal crypts* (Fig. 6) and they persist in unchanged number from the thirtieth day to the end of the experiment (sixty days).

Influence of Large Dosage of Heparin on Circulating Eosinophilia.

A certain amount of light may be thrown on the problem of the release of eosinophils *from the sources of their origin* by the study of the effects of large doses of heparin administered prior to the injection of eosinolytic hormones (hormones which precipitate circulating eosinopenia). This experiment was carried out on four men and five dogs. Each individual experiment was repeated twice (Table 4). In the first part of the experiment the eosinopenic hormone was given alone, and *this served as a control*. *In the second part of the experiment*, carried out four days later, heparin, in dosage much higher than required for the prevention of clotting, was administered intravenously ten minutes before the injection of eosinopenic hormone. Three dogs were injected with heparin alone. In the preliminary experiments (two dogs and one man) a low dosage of heparin, *i.e.*, sufficient to prevent *coagulation of* the blood (100 units per kilo), either did not influence the eosinopenia following the administration of adrenaline or gave no constant results.

The conclusions from these experiments are:

1. *The number of circulating eosinophils is associated in some way with clotting* (for details see pp. 75-76). The clotting process exercises a less marked influence on the circulating lymphocytes.

TABLE 4

Inhibition of Hormonally Induced Eosinopenia by Large Doses of Heparin.

No	Initial values: Circulating eosinophils/cu mm	Initial values: Circulating lymphocytes/cu mm	Initial values: Circulating polymorphs/cu mm	Initial values: Clotting time (min)	Heparin in anticoagulant (i u)	Clotting time 10 min after heparin injection	Hormone treatment (mg)	Values at end of 4th hour: Circulating eosinophils/cu mm	Values at end of 4th hour: Circulating lymphocytes/cu mm	Values at end of 4th hour: Circulating polymorphs/cu mm	Values at end of 4th hour: Clotting time
1 Man (a)	241	1,840	2,700	14	0	—	ACTH, 30	100	1,240	9,020	10 min.
(b)	135	1,353	2,337	12	125,000	> 4 hr	ACTH, 30	214	3,760	12,760	> 4 hr.
2 Man (a)	115	2,300	2,200	16 5	0	—	ACTH, 45	63	2,038	7,738	10 min
(b)	118	1,640	2,000	16	125,000	> 4 hr	ACTH, 45	152	5,320	9,570	> 4 hr.
3 Man (a)	106	2,700	2,385	6 5	0	—	Adrenal, 5	10	2,180	13,944	4 min
(b)	136	2,385	3,264	4	75,000	> 4 hr	Adrenal, 5	108	3,132	7,320	> 4 hr.
4 Man (a)	729	3,888	3,480	7	0	—	Adrenal, 5	260	3,900	20,280	5 min.
(b)	268	1,824	3,420	7	125,000	> 4 hr	Adrenal, 5	293	3,160	18,000	> 4 hr
5 Dog (a)	521	1,806	5,590	6	0	—	Cortisone, 20	390	1,300	10,400	4 min
(b)	304	1,380	6,716	7	15,000	> 4 hr	Cortisone, 20	759	4,600	16,100	> 4 hr
6 Dog (a)	1,312	4,100	9,840	5	0	—	Insulin, 50 units	483	1,539	13,680	3 min
(b)	738	4,235	11,300	4	30,000	> 4 hr	Insulin, 50 units	858	3,042	18,450	> 4 hr
7. Dog (a)	620	3,255	5,185	9	0	—	Insulin, 50 units	279	2,011	6,500	6 min
(b)	600	3,200	5 910	5	25,000	> 4 hr	Insulin, 50 units	545	3,640	13,280	> 4 hr
8 Dog	647	2,184	6,158	7	25,000	> 4 hr	0	847	2,914	5 264	> 4 hr
9 * Dog	32	2,140	7 918	8	25 000	> 4 hr	0	243	3,870	5,400	> 4 hr.
10 Dog	522	5,229	7,973	9	20 000	> 4 hr	0	860	5,332	10,492	> 4 hr

Rows (a) represent control and rows (b) experimental series

* The figures of the last four columns were obtained after the end of 24th hour.

2. Circulating eosinopenia is prevented by heparinisation of the blood *in vivo* (in proper dosage) when this precedes the injection of the eosinopenic hormones. In some cases heparinisation may produce even an increase in the number of circulating eosinophils.
3. The polymorphonuclear leucocytosis which follows the administration of these hormones is not affected by heparinisation.
4. The normal clotting time is slightly shortened by the administration of eosinopenic hormones.

Origin of Eosinophilisation of the Cryptal Epithelial Cells.

An understanding of how the cryptal epithelial cells of the intestine become eosinophilised may illuminate the problem of eosinophil formation in general For this reason various sources of this phenomenon are discussed below There are at least four possible explanations of the appearance of eosinophilic granules in the cryptal epithelial cells .

1. *Zymogenic Material as Source of Eosinophilisation of Intestinal Epithelial Cells* —It is well established that cryptal epithelial cells actively participate in the intestinal secretion (Verzàr *et al* , 1936 , and others) There are suggestions that this activity may be the source of the eosinophil granules in the epithelial cells , they might result from the retention of the zymogenic material in the resting stage of these cells, or the enzymatic material itself might form the eosinophilic granules inside the cytoplasm before it is eliminated during the active secretory stage

In an attempt to show the relationship between the secretory stages and the appearance of eosinophilic granules in the cryptal epithelial cells the following histological sections were prepared. Figs 7, 8, and 9 show the epithelial cells of a dog's jejunum in the resting phase. The dog, previously fed on raw meat for five days, was starved for forty-eight hours prior to the experiment. Fig 9 presents an intestinal crypt slightly distended, with large columnar epithelial cells containing big vacuoles of retained secretory material in their cryptal ends These cells of the resting stage show no resemblance to the eosinophilic granules seen in Figs. 7 and 8 of the same dog ; here eosinophilic epithelial cells in various stages of maturation are scattered

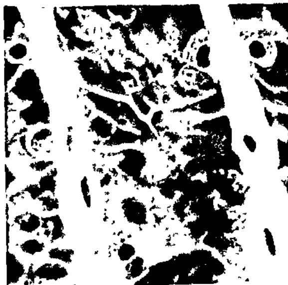


Fig 7

Biopsy of dog's duodenum on mixed diet · forty eight hours on water and dextrose. Plane of section cuts tangentially through the wall of the crypt, lumen of the crypt is not seen: artificial stratification created Various stages of maturation of eosinophilic epithelium Two neighbouring cells in central crypt in early stage of eosinophilisation. Two other cells are about to leave the cryptal wall Staining, azo-eosin. Magnification × 1000

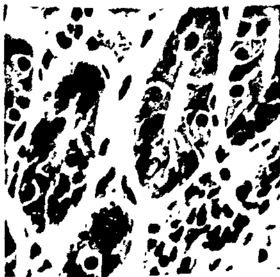


Fig 8

Biopsy of dog's jejunum on raw-meat diet; fasting conditions Specimen taken during third biopsy operation in a control animal Numerous eosinophilic cryptal epithelial cells in various stages of maturation with variable amounts of eosinophilic granulation in cytoplasm Two neighbouring cells in lateral crypt in later stage of developing eosinophilisation In lamina propria numerous lymphocytic cells and a few eosinophils Staining, azo eosin Magnification × 800.

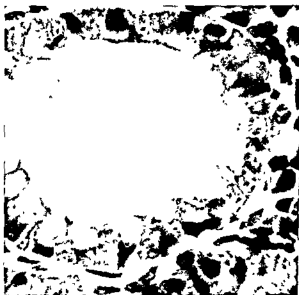


FIG 9

Dog's jejunum, raw meat diet for five days, in fasting condition, with secretory action in form of large vacuoles located at the end of the cell directed towards the lumen of the crypt No resemblance to the development of eosinophilisation. Staining, azo eosin Magnification × 1000

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irregularly throughout the whole length and thickness of the cryptal wall.

The example of intestinal epithelial cells in the secretory phase, shown in Figs. 5 and 6, is taken from a non-fasting mouse. The mouse has been chosen as the experimental animal because in the mouse the secretory cells and eosinophilic cells of the intestinal crypts are different types of cells bearing no resemblance to one another The secreted zymogenic granules are seen in the cryptal end of the secretory cells as well as in the lumen of the crypt , they are large, coarse granules localised always at the end of the cell nearest to the lumen of the crypt The cells containing these granules are grouped close together at the very bottom of the crypt . these are the Paneth cells, concerned probably in the zymogenic activity of the cryptal epithelium ; they are, however, distinctly different from the eosinophilised epithelial cells seen in Fig 6 in the same non-fasting mouse

Thus the suggestion that the secretory material of the intestinal cryptal cells is the source of the eosinophilic granules in the eosinophilised epithelial cells may reasonably be considered as invalidated.

2. *Blood Eosinophils as Source of Eosinophilisation of the Intestinal Epithelial Lining*.—The eosinophilised cryptal epithelial cells may be derived from altered blood eosinophils which have invaded the cryptal wall and subsequently adapted themselves morphologically to the local conditions This contention cannot be accepted for the following reasons

(a) Circulating eosinophils have completed their maturation ; a morphological transformation of the mature blood eosinophil into an *early* or *transitional* form of eosinophil, of size and shape similar to those of the adjacent columnar epithelial cells, is histologically inadmissible

Numerous immature eosinophils of mono-lobular type are present in the lamina propria outside the cryptal lining, at a time when the peripheral blood is entirely free from these forms.

If cryptal eosinophilised cells are derived from mature blood eosinophils it would be difficult to explain why they begin their structural transformation before entering the cryptal lining. On the

other hand, *early eosinophils*, formed locally and leaving the cryptal lining in their immature form, may become arrested in the lamina propria by the mechanism previously described (pp 35-74). The arrest of eosinophils moving from the crypts towards the interior explains the presence of immature eosinophils in the lamina propria while the circulating blood is entirely free from them.

(*b*) The nucleus of the eosinophilised cells in the epithelium in the early stages is always large, vesicular, and regularly rounded, containing threads of coarse chromatin By contrast the nucleus of the blood eosinophils is very densely filled with chromatin and characteristically bi-lobular. A transformation of bi-lobular into a mono-lobular nucleus cannot be easily accepted

(*c*) Figs 7, 8, and 10 show two adjacent eosinophilised epithelial cells. It is difficult to believe that these two cells could have penetrated to such a position in the cryptal wall by amœboid movements from outside the crypt, whereas the process of eosinophilic transformation may affect two neighbouring cells if the conditions for eosinophilisation exist in two adjacent cells.

Thus the myelogenous origin of the cryptal eosinophilised cells appears to be highly improbable

3. *Argentaffine Cells as Source of Eosinophilisation of Intestinal Epithelial Cells*—Argentaffine cells can easily be excluded as a source of eosinophilisation of the cryptal epithelial cells, since the gastric mucosa contains a great number of argentaffine cells and is absolutely free from *transitional eosinophils* (Figs 12 and 13).

4. *Local Formation of Eosinophils as Source of Eosinophilisation of Intestinal Epithelial Cells*—Another explanation of the appearance of the eosinophilic granules in the cryptal epithelial cells is **a local formation of eosinophils in the intestinal mucosa.** To substantiate this contention it is essential to discuss intestinal digestion of proteins in the light of the experiment with de-antigenisation of proteins in the diet (p. 66) and in the light of the theory of enzymatic adaptation of intracellular enzymes initiated by the invasion of the affected

Fig. 10

Duodenum of a dog on mixed diet, forty eight hours on water and dextrose. In the wall of central crypt two early transitional eosinophils in hydrated condition but retaining columnar shape. In the lateral crypt, partly seen, two neighbouring epithelial cells in later stage of the development of eosinophilia. In lumen of central crypt, two eosinophils in different stages of maturation. Fairly numerous lymphoid cells and eosinophils with mono lobular and bi lobular nuclei in lamina propria. Staining, azo-eosin. Magnification × 1000.

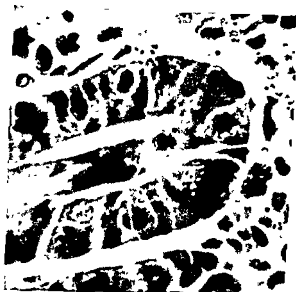




Fig. 11

Jejunum biopsy of dog No. 15 (Table 5) on mixed diet, forty eight hours on water and dextrose only. Biopsy removed two weeks after the initial operation. Intestinal crypts cut transversely, in two crypts are two different stages of epithelial eosinophil formation. (1) The earliest stage of eosinophilisation of the columnar epithelium with round nucleus and chromatin threads; eosinophilic mass begins to granulate, very fine vacuoles are present in the cytoplasm, the cell in a state of hydration. (2) An eosinophilic epithelium in the next crypt in a later stage, nucleus more condensed, deeply stained, pale cytoplasm with a few coarse eosinophilic granules. The shape of the cell is irregular with one pseudopodium—very numerous lymphoid cells and eosinophils with mono lobular and bi-lobular nuclei in lamina propria. Staining, azo eosin. Magnification × 800.

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Fig. 12

Dog's stomach on mixed diet; forty eight hours on water and dextrose Two argentaffine cells stained with azo-eosin showing fine reddish-brown granulations in cytoplasm, different from eosinophilic granulations of epithelium in intestine. No development of eosinophilisation in stomach gland epithelium Magnification × 1000



Fig. 13

Stomach of the same dog as in Fig. 12, stained by Jacobson's method. Seven argentaffine cells in close proximity Magnification × 1000

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cells by the unsplit protein molecules possessing antigenic capacity.

Unsplit protein molecules can be absorbed from a normal or pathological intestinal tract and cause various anaphylactic reactions (Verzár *et al.*, 1930) In **Part A** of the dietetic experiment described on page 63, ten days of an amino acid diet rapidly reduced cryptal eosinophilisation In **Part B** of the same experiment with the de-antigenised protein diet, transitional and mature eosinophils remained absent from the intestinal mucosa. They appeared there only when the same protein in its native form (not de-antigenised) had been supplied From this experiment it may be deduced that *the main factor which precipitates eosinophilisation of the cryptal epithelium is the antigenicity of the ingested proteins* The physico-chemical equilibrium of these cells is severely disturbed by the entry of foreign protein with antigenic power It is suggested that this disturbance leads to the appearance of eosinophilic granules in the cytoplasm.

Enzymatic Adaptation as Working Theory of Eosinophilisation.

The intracellular changes leading to eosinophilisation may be better understood in the light of the theory of enzymatic adaptation (Karström, 1938 Spiegelman 1950, and others) Details of this theory have been discussed in the chapter on "Sensitisation." The nucleus, stimulated by the intruding protein molecules, produces, as an adaptive enzyme, a new proteinase which can catabolise the foreign protein molecules (Fruton *et al*, 1941) In its anabolic phase the same adaptive enzyme can re-synthesise a new protein molecule (Bergmann, 1942) which in its structural pattern is identical with that of the invading molecule The newly formed replica of the foreign protein is next incorporated into the structure of the primarily invaded cell, because its molecules synthesised by the cell's own enzyme acquire the structural pattern of the cell's own proteins. In this way a cell invaded by antigenic molecules becomes a new biological entity, losing its original structure and function. One of the structural and functional transformations is the acquisition of amœboid mobility. Ultimately this cell moves away from the cryptal lining either into the lumen of the crypt (Fig. 10) or, more often, into the interior of

the villus (Fig 11) When it thus migrates, its antigenic property is carried with it (Godlowski, 1948 *b*).

Function of Lymphoid Barrier in the Lamina Propria Mucosæ of Intestine.

On the way into the interior of the organism this newly formed eosinophil must pass through the lymphocytic barrier of the lamina propria mucosæ. This lymphocytic barrier has been built gradually in the course of repeated invasions by various antigens from the lumen of the gut (Kuczynski, 1922). This barrier, through the various antibodies which it produces, serves as a defence wall against penetrating antigens from the alimentary canal (Kuczynski, 1922, Howell, 1928, McMaster *et al*, 1935, Ehrich *et al*, 1942) It may operate also by means of an antigen-antibody reaction in one of the forms previously described (p 5) The eosinophils containing a replica of antigenic protein in their cytoplasm enter the lamina propria, where they encounter a high local concentration of specific antibodies These antibodies penetrate the interior of the passing eosinophils and interact with the antigen therein As a result of this interaction the eosinophils become temporarily immobilised Thus those eosinophils which carry antigen are arrested in the lamina propria and remain immobilised there until the immobilising mechanism of the antigen-antibody reaction becomes exhausted

Mechanism of the Arrest of Eosinophils in the Intestinal Lymphocytic Barrier.

Blood eosinophils are either of *true* or of *pseudo* type (p 61), *true eosinophils* (i e, those formed in the bone-marrow) pass unaffected through the lamina propria mucosæ, causing no histo-chemical repercussions, because the intestinal lymphoid barrier possesses no specific antibodies against antigen carried by them. The *true* eosinophils may, on the other hand, possess an antigen which was incorporated in their structure in the bone-marrow, which, however, was brought to the bone-marrow by hæmatogenous spread from the absorptive surface of the alimentary canal. In this case the lymphocytic barrier of the lamina propria mucosæ may possess specific antibodies and, if these eosinophils incidentally are passing the intestinal mucosa

with the circulating blood, they may be arrested there by antigen-antibody reaction. This explains the appearance of mature eosinophils in the intestinal mucosa before the transitional forms of eosinophils were formed in the cryptal lining in mice which were fed on antigenic proteins (Tables 3A and 3B)

The *pseudo* eosinophils (*i e*, those formed in the non-hæmatopoietic organs—*e g.*, intestine), which were initially immobilised in the intestinal lymphocytic barrier and which subsequently regained their mobility in the final stage of the antigen-antibody interaction (p 76), can be rearrested in the zone which contains a high titre of locally accumulated antibodies in lymphoid tissue, provided that the eosinophils have in the meantime resynthesised the antigenic protein in their cytoplasm. The process of clotting appears to play an important part in the immobilisation of eosinophils That this is so is suggested by the fact that heparinisation of the blood, in concentration higher than that required for the abolition of clotting *in vivo*, prevents or significantly diminishes eosinopenia and lymphopenia, or in certain cases causes a rise in the circulating eosinophils and lymphocytes (see experiment with heparin on p 68). These fluctuations in the numbers of eosinophils and lymphocytes cannot be attributed to a technical error, since they were consistently found under the same experimental conditions, and they were also observed in similar conditions by Samter (1949)

The experiments with de-antigenised proteins in the diet and those with heparinisation *in vivo* suggest

1. That the primary mechanism which immobilises eosinophils in the intestinal mucosa is closely related to the antigen-antibody reaction
2. That the arrested eosinophils are in that stage of the antigen-antibody reaction in which cellular proteins are deprived of heparin

The process of de-heparinisation (in the initial stage of antigen-antibody reaction) resembles blood coagulation (precipitation), but it takes place in the cytoplasm of the affected cells. The main difference, however, between intracellular clotting and blood coagulation lies in the reversibility of the former process (Monnè, 1948). One can therefore postulate that the arrest of eos[illegible] in the lymphatic barrier of the

intestinal tract is due to the precipitation of the cytoplasmic proteins which follows the elimination of heparin; this in turn leads to loss of mobility of eosinophils As soon as this phase of the cellular reaction ceases, the cytoplasmic protein-coagulum within the eosinophils may become re-heparinised. The cell, thus regaining its mobility, is able to leave the lymphoid barrier Such a mechanism would also explain the mobilisation of eosinophils by a high concentration of heparin

Role of Heparin in Mobilisation of Eosinophils.

Heparin conjugated with proteins (Chargaff *et al.*, 1941) is present normally within the cells of all tissues (Charles *et al.*, 1933), and it ensures their semi-fluid state (Fisher, 1931). During anaphylactic shock the level of heparin in the blood is abnormally high (Jaques *et al*, 1941). The excess heparin is probably released from its conjugated form in the cells involved in the anaphylactic reaction

The mobilisation of eosinophils by a high concentration of heparin in the blood has been confirmed in an experiment in which an isolated loop of gut was perfused with highly heparinised blood Heparin in lower dosage, but in amounts sufficient to prevent coagulation, gave inconstant and variable figures for circulating eosinophils (Table 4) It is assumed, therefore, that heparin can penetrate the arrested eosinophils and restore the fluidity of the cytoplasmic proteins only when in high concentrations. The relatively small rise of eosinophils in the perfusion experiment (described on p. 89) was due to the perfusing of only a small area of intestine If, however, the whole organism was properly heparinised (dogs 7, 8, and 9 of Table 4), so that eosinophils were mobilised from the whole surface of the intestine, the increase in the circulating eosinophils was high enough to be statistically significant. Thus eosinophils regain their mobility and, reaching the blood, elevate the circulating eosinophil count. Further details of the heparin action on hormonally induced eosinopenia is discussed on page 99

Place of Origin of Eosinophils.

The above experiments suggest that medullary and extra-medullary eosinophil formation may normally take place

simultaneously. They also lead one to regard the mucosa of the small intestine as a normal source of eosinophilic cells in the blood; these, as *pseudo-eosinophils*, may contribute to the blood eosinophilia. The absorptive surface of the intestinal tract acts physiologically as a wide portal of entry for various antigens. During this physiological absorption, minute amounts of unsplit protein penetrate the cryptal epithelial cells and transform them into eosinophilic cells These cells thus carry various antigens whose invasion of the organism on a large scale is prevented by the defence mechanism of the intestinal lymphoid barrier (p 96)

The formation of *pseudo-eosinophils* in non-hæmatopoietic organs other than alimentary canal is conceivable on theoretical grounds, provided that a focus containing antigenic protein exists in the body Eosinophils which differ in origin possess two features in common their histological appearance and the biochemical mechanism of eosinophilisation by which they were formed. The type of protein which initiated the eosinophilisation may differ in eosinophils formed in different sources Thus there are at least two types of eosinophils in the circulating blood under normal conditions, namely, those formed in the bone-marrow and those formed in the alimentary canal, each type is genetically different and each type may carry a different antigen in its cellular proteins

THE FATE OF EOSINOPHILS IN EOSINOPENIA HORMONALLY INDUCED

INTRODUCTION

The problem of circulating eosinophil fluctuations has in recent years aroused a great interest in many research laboratories and in clinical departments mainly because these fluctuations have been observed to be closely correlated to adrenocortical activity, similar eosinophil fluctuations have been found to accompany the administration of those specific (ACTH) and non-specific (*e g*, insulin) stimuli which induce endogenous liberation of adreno-corticosteroids The close association of the circulating eosinopenia with the administration of exogenous ACTH led Thorn and his associates (1948)

to the elaboration of *the eosinopenic test* for the assessment of the physiological activity of suprarenals. Others suggested that the eosinopenic test may well be used for the assessment of the biological potency of various brands of corticosteroids with assumed eosinopenic action (Randolph, 1950, Speirs, 1952). Before accepting eosinophil diminution in the circulating blood as a morphological expression of cortical activity it must first be proved conclusively that this phenomenon results directly and solely from the action of corticosteroids If the eosinopenic activity involves intermediate secondary and tertiary factors, the conclusion from the eosinopenic test may be fallacious, because the intermediate factors may be primarily affected (for details see pp 75-99)

It is an undeniable fact that the injection of potent C^{11}-oxygenated corticosteroids is normally followed by eosinopenia, this eosinopenia lasts for so long as the other acute effects which resulted from the increased level of these corticosteroids are present After administration of a potent preparation of ACTH similar effects can be noticed plus other signs of adrenocortical stimulation, *e g.*, increased urinary output of 17-ketosteroids (Sprague *et al*, 1950, and others) Stimuli such as injection of adrenaline (Bertelli *et al.*, 1910, and others), insulin hypoglycæmia (Godlowski, 1946; and others), intraperitoneal injection of ammonium chloride in saturated solution (Josey *et al.*, 1932 *b*), intramuscular injection of sodium bicarbonate (Lawrence *et al*, 1932), injections of turpentine or nuclein (Staubli, 1910), as well as a great variety of infections (Zappert, 1893), parenteral administration of foreign protein (Staubli, 1910), starvation (Opie, 1904), intravascular hæmolysis (Meyer *et al*, 1909), internal hæmorrhage, hypertensive crisis, acute anoxia, colicky pain, and various chemical agents (Domarus, 1931), surgical procedures (Laragh. 1948), traumatic hæmorrhage (Recant *et al.*, 1950), and febrile conditions (Gabrilove, 1950) are among those stimuli which have been observed to cause eosinopenia. Finally, physical stimuli such as exposure to rapid changes of temperature (Josey *et al*, 1932 *a*), and various emotional reactions such as anger, fear, excitement, etc. (Speirs *et al.*, 1949), can also precipitate eosinopenia. It seems reasonable to describe all these influencing factors as stress met with by every living organism during everyday life (for details see "*Hormonal*

Involvement ") In other words, these pharmacological, immunological, nutritional, physico-chemical, and emotional stimuli which act upon an organism influence also circulating eosinophils and they do so by adrenocortical hormones liberated through the pituitary-adrenal response These complicated biochemical actions reflected in eosinophil fluctuations do not explain the role, the mechanism, and the meaning of the eosinopenia which follows.

METHOD

The following technique was applied in an attempt to elucidate the fate of eosinophils in hormonal eosinopenia

Eosinophil Counting.

An eosinophil count was made simultaneously by two methods ·

1 Randolph's counting-chamber method (1944).
2 Differential count and total white blood cells (WBC) count

Method (1) gives the white blood cells count and the *absolute eosinophil count.* Method (2) gives the number of eosinophils per 100 white blood cells The *absolute eosinophil count* by method (2) was determined by plotting the white blood cells and differential count The *circulating eosinophil count* was calculated by taking the average of the two figures obtained by these two methods This modification was called for because the other white blood cells reported in the present communication were calculated also in absolute numbers per cubic millimetre from the differential count and white blood cells count Most experiments were preceded, accompanied, and followed by such a *circulating eosinophil count*, otherwise it is specified which *count* was determined.

Assessment of Tissue Eosinophilia.

Tissue eosinophilia during hormonally induced eosinopenia was investigated both in human and in animal material at the end of the fourth hour after the eosinopenic stimulus was administered. The human material was obtained from normal

individuals during the administration of an adrenaline drip, or four hours after the symptoms of hypoglycæmia had appeared. Tissue from lungs (Godlowski, 1949), liver, spleen, and bone-marrow was obtained by needle aspiration biopsy An inguinal lymph node (with the adjoining subcutaneous tissue and skin) as well as a specimen of skeletal muscle was surgically removed.

Experiments were conducted on dogs wherever material for histological analysis of human organs was unobtainable Seventeen dogs were divided into four groups of these, three groups were treated and one group served as control The *eosinopenic action of ACTH * was investigated in the first* group (comprising three dogs), of adrenaline in the second group (comprising three dogs), and of insulin hypoglycæmia in the third group (comprising two dogs) The last group of nine dogs served as a control (Table 5)

Two other dogs were given adrenaline and killed four hours later, specimens of all organs were taken for sections to detect whether the eosinophils had been arrested during the circulating eosinopenia, and if so, where It was found that the intestinal mucosa was the only organ showing tissue eosinophilia, *attention was therefore directed to this tissue.* For this reason biopsies in survival experiments were taken only from the alimentary canal, mesenteric lymph node, and rib bone-marrow, the eosinophil count in rib bone-marrow films was made according to the recommendations of Best and Samter (1951) The findings in the mesenteric nodes and in the bone-marrow were persistently negative, therefore these results are not presented in the tables, and in further references to biopsies these organs are not mentioned The remaining organs were not obtained for histology until after death of the animal

All dogs, after a few days of preliminary observation and routine treatment for intestinal worms (Dicestal, M & B), were subjected to an initial laparotomy. During laparotomy biopsies were taken from the stomach, duodenum, jejunum, ileum, and colon Two or three weeks later the dogs of the experimental series were injected with eosinopenic hormones. At the end of the fourth hour the animals were either

* ACTH, Armour Standard LA-1-A, and Cortisone acetate in 1 5 per cent benzyl alcohol, Merck & Co., Inc, used in the present investigations, were supplied by the Medical Research Council, London, without charge

(1) re-laparotomised and alimentary canal specimens taken, or (2) killed and specimens of all organs removed for section. The animals which were re-laparotomised were kept for seven days, in order to recover from the effects of the injected hormones and from the operation itself Then they were killed and specimens of all organs were taken

The control group was treated in the same manner except for injection of eosinopenic hormones, thus the effects of the operation itself on the tissue eosinophilia of the intestinal mucosa was assessed

Laparotomies were performed under general anæsthesia (sodium thiopentone, Boots) In the first group two hypophysectomised dogs were injected intramuscularly with 10 mg. ACTH on the sixth day after hypophysectomy, and at the end of the fourth hour both dogs were killed

All dogs of treated and control groups were kept on water and dextrose for forty-eight hours prior to biopsy This measure was adopted to eliminate the influence of protein intestinal digestion on the histology of the mucosa whilst minimising starvation effects on the hypothalamico-pituitary-adrenal system (Opie, 1904)

The tissue specimens removed were fixed in Helly's modification of Zenker's solution, embedded in paraffin, sectioned and stained with azo-eosin. In cases treated with ACTH some sections were stained with azo-eosin and some by Jacobson's method for argentaffine cells (Jacobson, 1939)

Effects of Eosinopenic Hormones on White Blood Cells.

Direct action of eosinopenic hormones on white blood cells was investigated *in vivo* and *in vitro*.

1 **In vivo.**—The inferior vena cava (i v c) was ligated below the openings of the renal veins (Ligature 1). Both common iliac veins (c i v) were also ligated at their origin (Ligatures 2, *a* and *b*) Blood in one c i v. was separated from the rest of the vessel by another ligature placed at its junction with the i v c (Ligature 3) and served as a control. Through the wall of the other c i v a needle was inserted and all the blood from this *cava-iliac pocket* was aspirated into a *siliconned* syringe the desired amount of eosinopenic hormone was added to it Through the same needle the blood was reinjected into the vessel and another ligature (Ligature 4) placed before the

needle was withdrawn, the coagulation factors liberated from the damaged venous wall were thus isolated The *cava-iliac pocket* remained in the peritoneal cavity of the anæsthetised dog for two to three hours and the white blood cell analysis of both control and treated blood was made.

2. **In vitro.**—Heparinised blood (0·9 ml. of venous blood + 100 anti-coagulant units of heparin) was mixed with eosinopenic hormone Another sample of the same heparinised blood served as a control Both samples were placed in the incubator

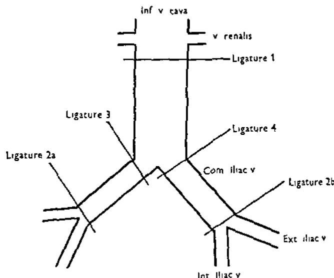


Fig 14

Cava iliac pocket, description in text

at 37° C for three hours and white blood cell analysis was made of both samples. The correction of the dilution factors in all calculations was made by the following formula.

$$\frac{\text{Number of cells found} \times \text{total blood volume (blood + hormone)}}{\text{Original blood volume}}.$$

This formula was applied to all experiments.

Intestinal Perfusion.

Intestinal perfusion was performed on four dogs under sodium thiopentone general anæsthesia A loop of *jejunum* was removed from the peritoneal cavity (vein and *artery*

ligated, leaving untouched nerve supply in the mesentery), and a segment of about 15 cm of it was perfused with the dog's own blood (200,000 anti-coagulant units per 200 ml of blood) at the rate of 100 drops per minute As soon as perfusion was established the perfused loop was returned into the peritoneal cavity. The perfusing blood in polythene tubing was warmed to 38° C in a water bath. The perfusion lasted two to four hours and was made in an ordinary transfusion set Oxygenation of the perfusing blood took place in the air through which the falling blood drops from the venous outlet of the tubing passed (a distance of 1 m.) to the collecting vessel, the change of colour of the venous blood to bright red proved that oxygenation took place In two dogs the effects of heparinised blood alone on intestinal eosinophilia was studied In another two dogs perfusion consisted of 200 ml of heparinised blood to which at the beginning of the perfusion 2 ml *Eucortone* (Allen & Hanburys Ltd) was added as eosinopenic hormone, a further 2 ml Eucortone was added at the end of the second hour of perfusion A biopsy from the jejunal loop outside the perfusion area was taken before the perfusion was started At the end of the perfusion biopsies were taken from the perfused area and also from the area outside the perfused loop The collection of samples of perfused blood for white blood cell analysis was made at hourly intervals from the venous end of the tubing, at the same time systemic blood samples were collected The morphological discrepancies between these two blood specimens proved the complete isolation of the two circulations

Hypophysectomy.

Hypophysectomy * carried out transpalatally was followed by electric cauterisation of the stalk After the death of the animal the stalk area was investigated by serial sectioning to discover whether the destruction of the pars tuberalis was complete (Pickford *et al.*, 1951).

Clotting Time.

Clotting time was determined by the glass capillary method.

* Hypophysectomy was performed by Dr M Pickford in the Department of Physiology, Edinburgh University.

Adrenaline Infusion and Insulin Hypoglycæmia.

Adrenaline infusions and insulin hypoglycæmia were used in men according to the technique described elsewhere (Godlowski, 1946). Adrenaline (0·3 mg per kilo of 0·1 per cent. aq. sol) was injected subcutaneously into dogs and supplemented by 1 mg of adrenaline in oil. Insulin hypoglycæmia was induced in dogs by subcutaneous injection of 5 units of soluble insulin per kilo. Hypoglycæmia has been terminated by intravenous injection of 20 to 40 ml of 20 per cent glucose solution followed by high carbohydrate diet.

RESULTS

Clinical Experiments.

Clinical experiments were carried out on five normal persons; *three were put on adrenaline drip and two were given insulin* hypoglycæmia Four hours afterwards biopsies were taken from the bone-marrow, lung, skin with subcutaneous tissue, lymph node, skeletal muscle, liver, and spleen. None of these biopsies showed eosinophils in sufficient number to explain the co-existing eosinopenia. These findings confirm the previously reported fact that eosinopenic hormones possess no eosinopenic action on the bone-marrow eosinophils (Godlowski, 1948 *a*).

Tissue Eosinophilia in Animals.

In the experiments with eosinopenic hormones *the influence* on tissue eosinophilia was studied in eight dogs, nine dogs served as control The result of the eosinopenic action of ACTH, adrenaline, and insulin are presented together, since they all work through the same mechanism, *i e*, *the liberation of* adreno-cortical steroids.

At the time of circulating eosinopenia the lamina propria mucosæ of the small intestine was riddled with mono-lobular and bi-lobular eosinophils (Fig 16). The data *presented in* Table 5 show the mean values of ninety High Power Field (HPF) counts of the tissue eosinophils with their Standard Deviation (SD). The term *early or transitional forms* refers to mono-nuclear cells of various shapes and sizes which contain

variable amounts of eosinophilic granules and eosinophilic masses These cells are, as a rule, localised in the lower parts of the crypts of Lieberkuhn The most immature forms of these cells have a columnar shape similar to the neighbouring epithelial cells (Figs 10 and 11) They are, however, usually larger than the adjacent cells in the epithelial lining They are four to five times bigger than mature blood eosinophils The nucleus of such a primitive cell is, in its initial stages, large and round, and contains coarse chromatin threads, in the later stages of maturation the nucleus becomes more compact (Figs 7, 8, and 11). The presence of many vacuoles in the cytoplasm gives this primitive cell an appearance of being hydrated (Figs 10 and 11), it thus stains more lightly than the adjacent cells of the epithelium Later still such a cell shrinks the eosinophilic granules condense and the cell approaches the pattern of a circulating eosinophil (Figs 7 and 8) The original columnar shape is lost as it acquires amœboid motility, and the nucleus gradually assumes an irregular shape (Fig 11). These transformations may occur in the cryptal epithelium, in the lumen of the crypt (Fig 10), or, most commonly, in the lamina propria mucosæ, therefore the lamina propria may contain mono-lobular and bi-lobular eosinophils

The columnar pattern of the deepest parts of the crypts is usually lost owing to the crowding of epithelial cells at the semicircular base of the crypt the part of the cells directed towards the lumen of the crypt may become very thin If the plane of the section cuts tangentially through this part of the crypt, certain cells—by pure artefact—may become separated from the cryptal lumen and so produce an artificial stratification Whenever this happens to an eosinophilised cell, one gets the erroneous impression that it is situated at the periphery of the crypt and that it has no direct contact with the cryptal lumen (Fig 7) This may be misinterpreted as being penetration of the cryptal wall from outside This point is worth stressing because in the discussion on the eosinophilisation phenomenon it plays an important role.

Argentaffine Cells in Alimentary Tract.

Abundant argentaffine cells (a c) (showing red-brown granulations when stained with azo-eosin as seen in Fig 12) are scattered throughout the whole thickness of the gastric

TABLE 5—*Eosinophil Levels*

Dog no	Initial eosinophil level, mean ±S D (cells/cu mm)	First biopsy: Organ	Tissue eosinophils/H P F ±S D: Transitional	Tissue eosinophils/H P F ±S D: Mature	2nd or 3rd week eosinophil level, mean ± S D (cells/cu mm)	Hormone administered
1	200 ±10	Stomach	0	1 ±0 30	350 ±0 6	ACTH, 10 mg
		Intestine	5 ±0 60	19 ±0 70		
		Colon	1 ±0 39	4 ±0 41		
2*	176 ±15	Stomach	0	1 ±0 25	200 ±10	ACTH, 10 mg
		Intestine	9 ±0 30	28 ±0 66		
		Colon	0	0		
3*	220 ±18	Stomach	0	0	280 ±7	ACTH 10 mg
		Intestine	3 ±0 04	14 ±0 06		
		Colon	0	1 ±0 02		
4	190 ±13	Stomach	0	0	201 ±5	Adrenaline, 3 mg
		Intestine	5 ±0 02	18 ±0 07		
		Colon	1 ±0 01	3 ±0 04		
5	242 ±10	Stomach	0	0	260 ±1 5	Adrenaline, 5 mg
		Intestine	7 ±0 01	41 ±0 60		
		Colon	2 ±0 02	5 ±0 02		
6	206 ±7	Stomach	0	3 ±0 03	285 ±5	Adrenaline, 5 mg
		Intestine	2 ±0 02	24 ±0 09		
		Colon	0	1 ±0 01		
7	960 ±12	Stomach	0	0	1100 ±12	Insulin 50 units
		Intestine	7 ±0 08	41 ±0 09		
		Colon	2 ±0 04	5 ±0 04		
8	190 ±5	Stomach	0	0	336 ±7	Insulin, 50 units
		Intestine	5 ±0 09	18 ±0 06		
		Colon	1 ±0 1	3 ±0 03		
9	251 ±7	Stomach	0	1 ±0 02	110 ±6	None control
		Intestine	4 ±0 05	20 ±0 09		
		Colon	2 ±0 02	4 ±0 05		
10	219 ±3	Stomach	0	2 ±0 01	190 ±3	None, control
		Intestine	5 ±0 03	32 ±0 07		
		Colon	1 ±0 03	4 ±0 06		
11	100 ±6	Stomach	0	0	151 ±5	None, control
		Intestine	2 ±0 03	5 ±0 08		
		Colon	1 ±0 04	1 ±0 05		
12	240 ±7	Stomach	0	1 ±0 01	635 ±10	None, control
		Intestine	6 ±0 03	20 ±0 30		
		Colon	4 ±0 07	2 ±0 08		
13	300 ±3	Stomach	0	1 ±0 01	303 ±9	None control
		Intestine	6 ±0 08	50 ±0 09		
		Colon	1 ±0 04	4 ±0 02		
14	1930 ±15	Stomach	0	1 ±0 05	1500 ±10	None, control
		Intestine	12 ±0 06	86 ±0 09		
		Colon	1 ±0 01	1 ±0 01		
15	630 ±9	Stomach	0	0	526 ±8	None control
		Intestine	8 ±0 07	33 ±0 09		
		Colon	2 ±0 01	4 ±0 02		
16	520 ±6	Stomach	0	0	900 ±4	None control
		Intestine	6 ±0 05	36 ±0 09		
		Colon	1 ±0 01	2 ±0 02		
17	684 ±7	Stomach	0	9 ±0 02	454 ±8	None, control
		Intestine	13 ±0 08	63 ±0 36		
		Colon	18 ±0 20	16 ±0 09		

The mean values of intestinal tissue eosinophilia were obtained from the readings of 90 High Power Fields (H P F) of tissue sections of duodenum, jejunum and ileum, 30 H P F of each.

mucosa Fig. 13 shows argentaffine cells (in a section from the same tissue block of the same animal as shown in Fig 12 stained by Jacobson's method) but no transitional eosinophils in the gastric mucosa In the small intestine in two adjacent sections of the same tissue block the number of argentaffine cells per high power field is ±2, whereas the number of transitional eosinophils is ±8.

nd after Hormone Treatment

Eosinophil level 4th hr after hormone (cells/cu mm)	4 hr after hormone treatment: Organ	Tissue eosinophils/H P F ± S D: Transitional	Mature	Eosinophil level 7 days after hormone action mean ± S D (cells/cu mm)	7 days after second biopsy: Organ	Tissue eosinophils/H P F ± S D: Transitional	Mature
65	Stomach	0	2 ± 0 05	320 ± 13	Stomach	0	1 ± 0 04
	Intestine	6 ± 0 04	48 ± 0 09		Intestine	9 ± 0 8	38 ± 0 09
	Colon	2 ± 0 08	6 ± 0 12		Colon	3 ± 0 09	8 ± 0 20
69	Stomach	0	1 ± 0 05	—	—	—	—
	Intestine	11 ± 0 37	50 ± 0 58				
	Colon	4 ± 0 40	7 ± 0 06				
100	Stomach	0	0	—	—	—	—
	Intestine	7 ± 0 05	18 ± 0 07				
	Colon	0	1 ± 0 04				
102	Stomach	0	0	—	—	—	—
	Intestine	3 ± 0 09	36 ± 0 07				
	Colon	1 ± 0 07	7 ± 0 05				
6	Stomach	0	0	—	—	—	—
	Intestine	6 ± 0 03	71 ± 0 10				
	Colon	0	0				
19	Stomach	0	1 ± 0 05	—	—	—	—
	Intestine	4 ± 0 03	22 ± 0 12				
	Colon	0	0				
130	Stomach	0	0	803 ± 17	Stomach	0	2 ± 0 09
	Intestine	8 ± 0 90	80 ± 0 07		Intestine	25 ± 0 13	70 ± 0 18
	Colon	6 ± 0 07	5 ± 0 05		Colon	7 ± 0 06	30 ± 0 61
10	Stomach	0	0	—	—	—	—
	Intestine	7 ± 0 08	33 ± 0 09				
	Colon	1 ± 0 60	4 ± 0 38				
—	Stomach	0	2 ± 0 06	—	—	—	—
	Intestine	3 ± 0 09	28 ± 0 10				
	Colon	0	1 ± 0 03				
—	Stomach	0	0	—	—	—	—
	Intestine	8 ± 0 05	43 ± 0 10				
	Colon	4 ± 0 08	3 ± 0 20				
—	Stomach	0	1 ± 0 06	—	—	—	—
	Intestine	4 ± 0 04	18 ± 0 04				
	Colon	1 ± 0 10	1 ± 0 09				
—	Stomach	0	1 ± 0 04	—	—	—	—
	Intestine	38 ± 0 19	41 ± 0 19				
	Colon	2 ± 0 09	2 ± 0 10				
	Stomach	0	5 ± 0 09	—	—	—	—
	Intestine	10 ± 0 14	70 ± 0 50				
	Colon	0	28 ± 0 14				
—	Stomach	0	0	—	—	—	—
	Intestine	20 ± 0 40	95 ± 0 09				
	Colon	4 ± 0 02	2 ± 0 06				
—	Stomach	0	1 ± 0 04	660 ± 22	Stomach	0	1 ± 0 07
	Intestine	12 ± 0 06	45 ± 0 11		Intestine	14 ± 0 08	59 ± 0 23
	Colon	1 ± 0 06	2 ± 0 04		Colon	1 ± 0 02	4 ± 0 07
—	Stomach	0	1 ± 0 01	600 ± 18	Stomach	0	1 ± 0 06
	Intestine	8 ± 0 10	37 ± 0 14		Intestine	8 ± 0 07	48 ± 0 18
	Colon	1 ± 0 20	1 ± 0 07		Colon	0	2 ± 0 06
—	Stomach	0	1 ± 0 03	576 ± 22	Stomach	0	10 ± 0 10
	Intestine	11 ± 0 17	55 ± 0 27		Intestine	29 ± 0 09	91 ± 0 33
	Colon	5 ± 0 06	10 ± 0 09		Colon	11 ± 0 10	40 ± 0 15

Values for stomach and colon were obtained in the same way S D = standard deviation.

* Dogs 2 and 3 were hypophysectomized.

Intestinal Eosinophilia related to Eosinopenic Hormones.

Tissue eosinophilia of the small intestine, at the time of blood eosinopenia, shows a significant rise in the number of eosinophils when compared with the initial biopsies (Figs. 15 and 16 and Table 5). The cryptal transitional eosinophils in the pre-experimental and experimental periods showed no

significant change. The intestinal eosinophilia of the post-experimental period was of the same degree as in the experimental period (dogs 1 and 7 of the treated series). The control animals showed an equal rise in intestinal eosinophilia during comparable periods (Fig. 17) Furthermore, the cryptal transitional eosinophils in the post-experimental periods of the treated and control series showed a significant rise in number per high power field despite the fact that there was *no concomitant circulating eosinopenia*

The conclusions from these experiments may be summarised as follows —

1. In the epithelium of the intestinal crypts there are cells with eosinophilic granules which morphologically can be regarded as early forms of blood eosinophils.
2. The increased eosinophilia of the intestinal mucosa in *the experimental and in the control periods should be* considered as a result of repeated trauma of the operation itself These traumatising stimuli intensify the process of local formation of eosinophils and mobilise the mechanism whereby eosinophils, which are moving from the cryptal lining towards the interior of the organism, are arrested in the lamina propria mucosæ The arrest of eosinophils in the lamina propria, however, does not prevent the return of circulating eosinophils to the previous level.
3. Eosinopenic hormones which exhibit their action on the circulating eosinophils do not maintain their eosinolytic activity on the tissue eosinophils
4. Argentaffine cells are histological elements unconnected with the transitional eosinophils of the cryptal epithelium

Intestinal Perfusion.

The perfusion experiment was carried out in two groups of two dogs each The data obtained are presented in Tables 6A and 6B.

In Part A a loop was perfused with 200 ml. of the dog's own heparinised blood. In the systemic blood the white blood cell count was significantly increased in two hours, the increase being due to circulating polymorphonuclear leucocytes.



Fig 15

Jejunum biopsy of dog No 1 (Table 5) removed three weeks before the experiment. Circulating eosinophilia, 179 per cub mm Two intestinal crypts in one crypt an early eosinophil with mono-lobular nucleus, dense eosinophilic granulations in cytoplasm One mono lobular eosinophil in interior of the villus and one mono-lobular eosinophil in the lumen of the crypt Four eosinophils and a few lymphoid cells in lamina propria mucosæ Staining, azo eosin. Magnification × 800

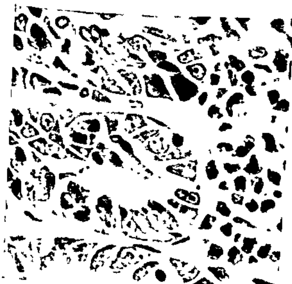


Fig 16

Jejunum biopsy of the same dog removed three weeks after first biopsy at the end of fourth hour after injection of 10 mg of ACTH, circulating eosinopenia, 60 per cub mm In the central crypt four transitional granulated eosinophilic cells with mono lobular nuclei in lamina propria numerous lymphoid cells and numerous eosinophils with mono lobular as well as bi lobular nuclei Staining and magnification as in Fig 15

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Fig 17

Jejunum biopsy of the same dog taken seven days after the experiment presented in Fig 16 Circulating eosinophilia, 329 per cub mm Very numerous eosinophils with mono lobular and bi lobular nuclei and very numerous lymphoid cells in lamina propria Staining and magnification as in Fig 15

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	0 min		60 min		120 min		180 min		240 min	
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Systemic blood										
W B C /cu mm	6,110	9 500	11,500	13,266	11,600	16,000	—	—	—	—
Eosinophils/cu mm	620	1,045	230	536	232	480	—	—	—	—
Polymorphs/cu mm	3,030	5,700	8,305	8,710	8,120	11,360	—	—	—	—
Lymphocytes/cu mm.	2,220	2,565	2,530	3,618	2,784	3,520	—	—	—	—
Monocytes/cu mm	240	190	345	402	464	640	—	—	—	—
Perfusing blood:										
W B C /cu mm	6,110	9,500	4,600	8,500	6,356	8,440	—	9,500	—	9,200
Eosinophils/cu mm	620	1,045	460	1,020	701	1,385	—	1,235	—	1,104
Polymorphs/cu mm.	3 030	5,700	2,070	4,930	2,600	4,505	—	5,415	—	5,336
Lymphocytes/cu mm.	2,220	2,565	1,840	2,295	2,795	2,465	—	2,660	—	2,576
Monocytes/cu mm	240	190	230	255	260	85	—	190	—	184
Tissue eosinophil count/H P F										
Jejunal mucosa before perfusion	51–61 (60)	40–87 (55)	—	—	—	—	—	—	—	—
Jejunal mucosa after perfusion	—	—	—	—	23–42 (30)	—	—	—	—	16–32 (23)
Control non perfused jejunum	—	—	—	—	44–75 (58)	—	—	—	—	76–84 (80)

TABLE 6B.—*Perfused Jejunum of Dog with Heparinised Blood + 2 ml Eucortone (1 per cent)*

	Dog 3	Dog 4	Dog 3	Dog 4	Dog 3	Dog 4	Dog 3	Dog 4	Dog 3	Dog 4
Systemic blood:										
W B C /cu mm	6,060	10,083	5,000	12,612	7,661	12,809	15,400	—	19,970	—
Eosinophils/cu mm	240	183	50	173	38	37	0	—	20	—
Polymorphs/cu mm	4,140	8,200	4,050	10,414	7,315	9,288	14,168	—	18,000	—
Lymphocytes/cu mm.	1,260	1,400	450	1,771	154	2,709	616	—	975	—
Monocytes/cu mm	420	300	450	254	154	775	616	—	975	—
Perfusing blood:										
W B C /cu mm	6,000	8,800	2,760	7,828	2,100	8,000	2,100	7,948	2,300	7,923
Eosinophils/cu mm	240	114	31	106	42	60	21	47	23	23
Polymorphs/cu mm	3,840	7,216	2,174	7,216	1,848	6,880	1,890	6,952	2,001	7,031
Lymphocytes/cu mm	1,500	1,149	262	738	105	880	105	711	138	790
Monocytes/cu mm	420	326	293	168	105	160	84	238	138	79
Tissue eosinophil count/H P F:										
Jejunal mucosa before perfusion	32–76 (51)	33–83 (55)	—	—	—	—	—	—	—	—
Jejunal mucosa after perfusion	—	—	—	—	—	—	—	—	17–29 (25)	32–40 (36)
Control non perfused jejunum	—	—	—	—	40–54 (50)	53–93 (62)	—	—	—	—

Within the same time eosinopenia developed. Both these phenomena resulted from the stress of the operation and general anæsthesia (Laragh, 1948).

In the perfusing blood a slight eosinophil increase was worth noticing, even though by itself this elevation was too small to be significant. The tissue eosinophilia of the perfused jejunal mucosa showed a significant decrease during that same period.

In Part B a jejunal loop was perfused with 200 ml. of the dog's own heparinised blood to which Eucortone had been added The systemic blood showed the same findings as in Part A In the perfusing blood the total white blood cell count was significantly decreased only in one dog (No 3) This was the result of a great reduction in eosinophils, lymphocytes, polymorphs, and monocytes (Tables 6A and 6B). All granular groups of white blood cells showed conspicuous degranulation and loss of staining affinity, with vacuolisation of the cytoplasm The nuclei showed marked regressive changes in the form of pyknosis and karyorrhexis. The lymphocytes and monocytes exhibited similar changes. The red blood cells did not show any significant signs of damage due to the altered osmotic pressure

At the end of the perfusion the eosinophil count per high power field of a section from the perfused area of the jejunum was significantly reduced.

The conclusions drawn from the perfusion experiment are·

1 The stress of the operation itself precipitates changes in the systemic blood which are characterised by polymorphonuclear leucocytosis and eosinopenia.
2. During the perfusion the heparinised blood shows a slight eosinophil increase. This small rise in number of eosinophils per cubic millimetre is not significant *per se*, but taken in conjunction with the significant diminution in the tissue eosinophilia of the perfused jejunum it may be regarded as a result of the mobilising effect of heparin on the tissue eosinophils; the smallness of the perfused area from which oesinophils were mobilised explains the slight increase in number of eosinophils in the perfusing blood.
3. The perfusing heparinised blood with Eucortone added causes a great diminution in the circulating eosinophils, polymorphonuclears, lymphocytes, and monocytes.

All white blood cells in these experimental conditions show regressive changes in both cytoplasm and in nucleus, and these changes take place in the blood stream.

4. The final quantitative and qualitative changes in the white blood cells in the perfusing blood containing eosinolytic hormone result from two independent actions :
   (a) The mobilisation of eosinophils by heparin from the perfused area
   (b) The cytolytic action exerted by eosinopenic hormone on all white blood cells in the perfusing blood

The Direct Action of Eosinopenic Hormones on White Blood Cells.

The direct action of eosinopenic hormones on white blood cells was investigated in two separate experiments *in vivo* and *in vitro*

In vivo (Table 7).—Eosinophils were the leucocytes most affected by the leucolytic hormones ; next in susceptibility came the monocytes and lymphocytes, and the least affected were polymorphonuclear leucocytes Many of the white blood cells showed the features of disintegration described in the perfusion experiment. Blood samples mixed with desoxycorticosterone acetate (DCA) did not exhibit similar quantitative or qualitative changes in their white blood cells The effectiveness of the cytolytic action in the isolated blood *in vivo* was related to the concentration of the administered hormone and to the duration of its action.

In vitro (*Table 8*).—Eosinopenic hormone mixed with heparinised blood also caused significant diminution in eosinophils, whereas lymphocytes, monocytes, and polymorphonuclear leucocytes were only slightly affected. The cytolytic action was also related to the concentration and the length of time of the action of the hormone administered.

The conclusions from these experiments are :

1. Eosinopenic hormones possess a direct cytolytic action on white blood cells under the experimental conditions applied.

TABLE 7

Venous Blood isolated in Blood Vessels in vivo *not treated with Anticoagulant + Hormones causing Eosinopenia*

No	Preliminary differential count, absolute values/cu mm					Amount of hormone/ml blood	Time of experiment (hr.)	Differential count in experimental period, absolute and corrected values of the treated blood					Differential count in experimental period of non treated blood after 3 hr, absolute values/cu mm in isolated blood vessel				
	Poly morphs	Eosino phils	Lym phocytes	Mono-cytes	Leuco-cytosis			Poly-morphs	Eosino-phils	Lym-phocytes	Mono cytes	Leuco-cytosis	Poly morphs	Eosino-phils	Lym phocytes	Mono cytes	Leuco cytosis
1. Dog	5000	1155	2639	273	9067	Eucortone, 0 15 ml.	1	6720	576	2016	288	9600	4134	1014	2418	234	7800
2. Dog	5320	501	1444	304	7569	Eucortone, 0 15 ml	2	5806	275	1451	426	7958	5220	435	2436	609	8700
3. Dog	3770	348	1276	406	5800	Eucortone, 0 20 ml	2	2080	52	364	104	2600	3212	290	748	170	4426
4. Dog (*a*)	5655	783	1914	348	8700	Cortisone acetate, 100 μg	2	2900	290	2552	58	5800	4004	847	2618	231	7700
(*b*)	—	—	—	—	—	DCA, 0 50 mg	2	4092	614	1716	108	6620	—	—	—	—	—

Hormone in vitro

No	Preliminary differential count, absolute values/cu mm: Poly morphs	Eosino phils	Lym phocytes	Mono-cytes	Leuco-cytosis	Adrenal hormone/ml blood	Differential count of treated blood kept for 3 hr in 37° C: Poly-morphs	Eosino-phils	Lym phocytes	Mono cytes	Leuco-cytosis	Differential count of heparinized blood kept for 3 hr in 37° C: Poly-morphs	Eosino-phils	Lym-phocytes	Mono cytes	Leuco-cytosis
1. Dog	3,074	348	2,030	336	5,788	Eucortone, 0 05 ml	4,173	112	1,400	224	5,907	3,250	250	1,300	200	5,000
2 Dog	10,428	474	4,108	790	15,800	Eucortone, 0 05 ml	11,020	145	2,610	725	14,500	11,076	384	1,556	284	13,300
3. Dog	8,460	1,170	3,666	705	14,001	Eucortone, 0 1 ml	5,994	444	4,440	222	11,000	5,405	849	4,945	345	11,544
4 Man	5,025	300	1,875	300	7,500	Eucortone, 0 15 ml	3,680	126	644	230	4,690	4,624	272	1,572	340	6,808
5 Dog	8,470	333	1,430	770	11,003	Eucortone, 0 2 ml	7,030	95	2,280	95	9,500	8,280	360	3,000	360	12,000
6 Man (*a*)	3,717	135	1,888	177	5,917	Cortisone acetate 5 µg	3,096	83	989	120	4,297	3,328	124	1,664	104	5,220
(*b*)	—	—	—	—	—	Cortisone acetate 50 µg	3,024	48	1,536	172	4,780	—	—	—	—	—
(*c*)	—	—	—	—	—	Cortisone acetate, 100 µg	2,930	0	1,026	114	4,070	—	—	—	—	—
(*d*)	3 530	150	1,432	104	5,222	Cortisone acetate, 1 mg	3,055	47	1,410	188	4,700	3,536	134	1,352	104	5,126
(*e*)	—	—	—	—	—	Cortisone acetate, 2 mg	3,312	96	1,248	144	4,800	—	—	—	—	—
(*f*)	—	—	—	—	—	Cortisone acetate, 3 mg	2,484	10	972	108	3,574	—	—	—	—	—
(*g*)	—	—	—	—	—	Cortisone acetate, 4 mg	2,015	18	930	124	3,097	—	—	—	—	—
(*h*)	—	—	—	—	—	Cortisone acetate, 5 mg	2,400	21	910	119	3,450	—	—	—	—	—
(*i*)	3,717	131	1,888	177	5,917	DCA, 1 mg	4,012	118	1,543	236	5,909	3,328	124	1,064	104	5,220
(*j*)	—	—	—	—	—	DCA, 2 mg	4,015	101	1,210	220	5,548	—	—	—	—	—
(*k*)	—	—	—	—	—	DCA, 3 mg	3,432	101	1,080	104	5,617	—	—	—	—	—
(*l*)	—	—	—	—	—	DCA, 4 mg	3,276	108	1,812	208	5,204	—	—	—	—	—
(*m*)	—	—	—	—	—	DCA, 5 mg	3,920	168	1,344	168	5,600	—	—	—	—	—

2. The destructive action of these *hormones affects all* white blood cells, polymorphonuclear leucocytes, lymphocytes, and monocytes being less susceptible to this action than eosinophils.
3. Involutionary changes in all white blood cells were present in both experiments described.

DISCUSSION

Physiological Fluctuations in Circulating Eosinophils.

In recently published reports (Rud, 1947 ; leading articles, *Lancet*, 1950, *a* and *b* ; and others) doubts have been expressed about the accuracy of the methods at present in use for the counting of eosinophils The main objection stressed is the great variation in counts of circulating eosinophils, even in normal conditions, which makes it impossible to establish a base line for their evaluation These difficulties, which doubtless exist, result chiefly from the fact that the counts of circulating eosinophils fluctuate significantly even in physiological conditions, such fluctuation is one of the basic phenomena of eosinophil function, this phenomenon has been termed *endogenous eosinopenia* (Halberg, 1952)

Adrenaline (in suitable dosage and administered in an appropriate way) precipitates eosinopenia by mobilising the pituitary-cortical response, the chemical and morphological results can be detected in from ten to twenty minutes after intravenous injection, or a little later after subcutaneous injection (Godlowski, 1948 *a*, and others) Adrenaline liberated from the adrenal glands of the organism itself acts far more quickly (Vogt, 1950) On the other hand, dissipation of adrenaline from the suprarenal medulla takes place easily during everyday life by the *run and fight* mechanism described by Cannon, which in recent terminology is designated as *sympathetico-adrenal discharge* Speirs *et al* (1949) have shown that a drop in eosinophils occurs after simple handling of experimental animals, and that this fall is caused by liberation of adrenaline from the adrenal medulla, this eosinopenia is as great as that following injection of ACTH or adrenaline itself.

It has been shown (Halberg, 1952, Higgins, 1952) that *endogenous eosinopenia* occurs physiologically and is characterised by a *twenty-four-hour periodicity* cycle. This eosinopenic

rhythm is species specific, and this species specificity depends on the initiation of vital activities as well as on the action of light Thus in humans and in dogs (diurnal type of life) the lowest count of eosinophils occurs in the early hours of the morning; in mice and rats (nocturnal type of life) it occurs in the early hours of the night This physiological rhythm in the circulation of eosinophils is controlled primarily by the action of the autonomic nervous system, the endocrinological response being secondary.

It is worth mentioning that a post-adrenaline eosinopenia in hypophysectomised or adrenalectomised animals does occur, but its origin is not clearly understood It has been found that in adrenalectomised dogs (Erdstein *et al* 1945) and in hypophysectomised rats (Fry, 1952) and mice (Speirs, 1952 and others) a transitory eosinopenia takes place after the injection of adrenaline, this type of post-adrenaline eosinopenia is non-specific in character and varies greatly from one species to another. The suggestion that adrenaline in these circumstances causes sequestration of eosinophils in the capillary bed of various organs (*e g*, lungs, liver, spleen) by its action on the autonomic nervous system has been disproved in men and in dogs by the experiments previously described (p 84) The post-adrenaline eosinopenia of adrenalectomised or hypophysectomised laboratory animals may be explained by the following coincidental factors (1) The existence of additional adreno-cortical tissue elsewhere in the body, (2) regeneration of the removed organs, or (3) stimulation of other organs by adrenaline to produce steroids with mild eosinolytic action (*e g*, gonads, Fry, 1952)

In other words, the great variations in circulating eosinophils found in physiological conditions are the result of a very sensitive and rapidly operating eosinopenic reaction in which the autonomic nervous system, glandular part of the pituitary gland, and suprarenals are involved. the autonomic nervous system is, however, the primary link in this chain of physiological reactions.

Hormonal Eosinopenia and Tissue Eosinophilia.

In the experiments already described, in which eosinopenic stimuli were applied in the form of three different hormones, the results obtained were almost identical One could have

anticipated this, since all three hormones stimulate (directly or indirectly) the secretion of those adreno-cortical steroids which are responsible for circulating eosinopenia. The mechanism and the meaning of this action is not clear and needs elucidation

ACTH does not exert its eosinopenic action unless the cortex of at least one adrenal gland is able to secrete steroids in sufficient amount to eliminate eosinophils from the circulation (Thorn *et al*, 1948). But there is no general agreement as to which steroids possess an eosinopenic action. Compounds E and F, the amorphous fraction of cortical extracts (Well *et al*, 1940, Engstrom, 1948, Fourman *et al.*, 1950 Higgins, 1952; and others), and whole gland extracts unquestionably possess this activity. Desoxycorticosterone acetate exhibited very weak eosinopenic as well as leucolytic action in the experimental conditions described elsewhere in this essay

The histological results of the series of experiments presented here point to the mucosa of the small intestine as the only tissue in the dog which contains a significantly high number of eosinophils during hormonal circulating eosinopenia The question now arises as to whether circulating eosinophils are arrested in the intestinal mucosa and thereby removed from the circulation causing eosinopenia The answer to this question lies in a closer analysis of the fluctuations in tissue eosinophilia of the small intestine Eosinophils were found in the intestinal mucosa in the experimental periods in much higher numbers than during the pre-experimental periods On the other hand the readings of tissue eosinophilia in the post-experimental periods and in the non-treated controls were either equal to or higher than those of the experimental periods, while the number of circulating eosinophils remained within normal limits (Table 5) In other words, the high intestinal eosinophilia of the experimental period cannot be regarded as resulting from the arrest of the blood eosinophils, with consequent eosinopenia in the blood *The local accumulation of eosinophils in the mucosa and the contemporary blood eosinopenia are two unrelated phenomena*; the increasing intestinal eosinophilia is caused by accelerated formation of eosinophils in the mucosa and their arrest in the lymphatic barrier of the lamina propria while moving from the crypts into the interior of the organism (for mechanism see p. 75). Both these factors, *i e*, the forma-

tion and the arrest of eosinophils, are aggravated by the repeated traumatisation of the intestinal mucosa by biopsies The blood eosinopenia, coinciding with the tissue eosinophilia, cannot therefore be regarded as resulting from the arrest of circulating eosinophils in the intestinal mucosa, its cause must be sought in the direct action of the corticosteroids on eosinophils by the mechanism which is described in detail on page 58.

The increasing tissue eosinophilia in the traumatised organs during the coexisting hormonal eosinopenia in the blood points to the difference between tissue and blood eosinophils so far as their susceptibility to the eosinolytic action of corticosteroids is concerned. The tissue eosinophils are persistently resistant to the eosinolytic action of corticosteroids at a time when the circulating eosinophils are highly susceptible (Godlowski, 1952*a*) This observation has been confirmed also by others (Gordon, 1952; Muehrke, 1952, Speirs, 1952) In an attempt to explain the resistance of tissue eosinophils to the hormonal lytic action it will be well to examine the arrest of eosinophils in the intestinal mucosa more closely (for details see p 75) The arrest of eosinophils in the lymphoid tissue which surrounds the portal of entry of the antigen (carried by eosinophils) is performed by an antigen-antibody reaction in the following manner the gelation of the cytoplasm of eosinophils, which occurs during the antigen-antibody reaction (antigen of eosinophils and antibody released from the lymphocytes of the lymphoid barrier, for details see p 74), immobilises the eosinophils and makes them impenetrable by the corticosteroids Until the cytoplasm of the eosinophils has been made fluid again by heparin (p. 76) the corticosteroids cannot enter them and are therefore unable to initiate protein catabolism, which is ultimately responsible for the destruction of eosinophils. But when their cytoplasm becomes fluid again the eosinophils regain their motility and move away from the tissue in which they were arrested; at the same time they become penetrable by the corticosteroids and may be destroyed by the accelerated protein catabolism which follows this penetration

Direct Action of Eosinopenic Hormones on White Blood Cells.

Experiments to test the direct action of C^{11}-oxygenated corticosteroids on white blood cells *in vivo* and *in vitro*

(Tables 6A and 6B, 7, and 8) have shown that a very high dosage of cortisone acetate is required to perform the destructive action. These experiments have also confirmed the finding that corticosteroids destroy eosinophils in the blood stream but not in the tissues (discussed in the preceding paragraph). The concentration of cortisone in these experiments was much higher than is ever found under natural conditions. It seems that such massive dosage is necessary to compensate for the artificial *milieu* of the experimental conditions described. Since the involution of all white cells caused by leucolytic corticosteroids is both marked and rapid (confirmed also by Padawer *et al.*, 1952), one can reasonably postulate that the involution is due to the catabolic phase of intracellular protein metabolism. The alternative suggestion that the anabolic phase is inhibited (Green, 1950, and others) seems less attractive because anabolic inhibition would require a much longer time to produce the degree of destruction seen in the presented experiments.

The susceptibility of white blood cells to this action under experimental conditions diminishes in the following order: *eosinophils, monocytes, lymphocytes, and polymorphonuclears. It is suggested that eosinopenia following the application of a potent eosinopenic steroid is due to the disintegration of eosinophils in the blood as a result of mediation of the eosinopenic steroids in the protein metabolism leading to a rapidly progressing protein degradation.* Disintegration of the nuclei and cytoplasm of all types of white blood cells resulting from the accelerated protein catabolism can be seen in a blood film. The coexisting mobilisation of polymorphonuclear leucocytes may mask the cytolysis of these cells (see next paragraph). By analogy one can expect acceleration of the catabolism of cellular proteins in other tissues whose enzymatic structure is similar to that of white blood cells (for details see "Anaphylactic Reaction"). This contention finds valuable support in the negative nitrogen balance seen in clinical cases of hypercorticism and in treatment with a high dosage of cortisone or ACTH.

Muehrke *et al.* (1952) find that heparin mixed with the blood *in vitro* inhibits the destruction of eosinophils caused by C^{11}-oxygenated corticosteroids. They do not report the effects of these steroids on other white blood cells, nor do they offer any explanation of the inhibiting action of heparin in their experimental conditions.

The inhibiting action of heparin on the eosinolytic activity of corticosteroids may be illuminated by discussing two findings reported independently (1) Inhibition by heparin of the hydrolytic action of trypsin at *p*H 7 3 through the formation of a trypsin-heparin complex, this complex may be dissociated by acidifying to *p*H 3 0 (Fisher *et al*, 1937, Rocha e Silva *et al*, 1945). (2) The destructive action exerted by corticosteroids on eosinophils by means of a rapid proteolysis of their cellular proteins (Godlowski, 1952 *a*) In the experimental conditions applied by Muehrke and his co-workers, heparin inactivated the tryptic enzymes in the blood, thus corticosteroids could not initiate proteolysis in eosinophils despite the disruption of the lipo-protein molecules (p 51) and despite the exposure of their cellular proteins to hydrolysis, because the proteolytic enzymes were blocked by heparin This mechanism is unlikely to operate *in vivo* unless and until a very high dosage of heparin (p 68) has temporarily inactivated all the available active proteinase in the organism This independent observation of Muehrke and his co-workers is very valuable because it supports the suggested contention that eosinolysis, when caused by corticosteroids, is primarily a proteolysis

In the prevention of hormonal eosinopenia by heparin, two independent factors are involved.

1. The inhibition of proteolytic enzymes which are actively destroying the eosinophils of the circulating blood
2. The mobilisation of the tissue eosinophils.

Heparin when injected intravenously in high dosage causes a significant increase in circulating eosinophils (dogs 8, 9, and 10 (Table 4)) When in a perfusion experiment it is added to the perfusing blood a significant diminution of the tissue eosinophil count of the perfused organ results, together with a simultaneous but slight increase in the eosinophil number in the perfusing blood (Tables 6A and 6B)

In the light of this discussion the eosinopenic test (Thorn *et al.*, 1948) must be regarded as an insufficiently reliable test for the biological activity of C^{11}-oxygenated corticosteroids if it is not accompanied by evaluation of the heparin level in the blood. Heparin in high concentrations prevents hormonal eosinolysis even in the presence of fully active corticosteroids Hence the conclusion that corticosteroids and the suprarenal

glands are not the only factors which may significantly influence the eosinophil count in the eosinopenic test.

Mechanism of Polymorphonuclear Leucocytosis following Injection of Corticosteroids.

Menkin's (1950) work on the biochemical composition of inflammatory exudates explains (in some measure) the leucocytosis which follows the administration of C^{11}-oxygenated corticosteroids, a leucocytosis which masks the simultaneous destruction of polymorphonuclear leucocytes (see also pp. 58, 88). A close analysis of the fluctuations which occur in the polymorphonuclear leucocyte count during perfusion experiments (Tables 6A and 6B) strongly suggests that Menkin's findings are applicable in these conditions.

In the experiment in which a dog's intestine was perfused with his own blood to which Eucortone had been added, the polymorphonuclear leucocytes (in dog No 3) were rapidly reduced in number The masking action could not take place in the perfusing blood because the perfusing circuit was cut off from the source from which polymorphonuclear leucocytes would have been mobilised But the systemic blood, at the same time, showed a steady rise of polymorphonuclear leucocytes (this blood remained under the action of the corticosteroids liberated from the dog's own suprarenals by the stress of the operation and general anæsthesia) Comparing at hourly intervals the leucocyte figures of the perfusing blood with those of systemic blood, one finds a reduction of polymorphonuclear leucocytes in the former and a rise in the latter.

Menkin isolated from inflammatory exudates a substance which he designated *leucocytosis promoting factor* which is derived from the cells participating in the inflammatory reaction. Since polymorphonuclear leucocytes are the most characteristic and numerous cells participating in inflammation, it is conceivable that they contribute substantially to the production of the *leucocytosis promoting factor*

These two independent findings can be correlated with one another and may explain the mechanism of polymorphonuclear leucocytosis following the administration of C^{11}-oxygenated corticosteroids. As a result of the catabolic action of these corticosteroids, polymorphonuclear leucocytes disintegrate and

they release *the leucocytosis promoting factor*; this in turn mobilises polymorphonuclear leucocytes from the bone-marrow to such an extent that the coexisting polymorpholysis is completely masked and leucocytosis supervenes

FUNCTION OF EOSINOPHILS

Summary of Eosinophil Formation.

Any living cell, whose intracellular enzyme system has become changed in such a way that invading protein molecules are no longer split, may turn into an eosinophil This change seems to be related to the physiological or pathological *ageing* of the enzyme system The foreign protein molecules remaining unaltered in the interior of such a cell stimulate the nucleus to produce a new proteinase or to reorganise the existing debilitated proteinases (by a process of adaptation of enzymes) in such a way that the foreign protein can then be catabolised. This reorganised enzyme in its anabolic activity may re-synthesise a protein molecule which in its pattern is a replica of the original invading molecules (Bergmann, 1942) This newly reproduced protein molecule, as manufactured by the cell's own enzymes, is incorporated into the structure of the cytoplasmic proteins Such a possibility in the formation of eosinophils is supported by the recent histo-chemical study of eosinophils by Vercanteren (1951) In this way the debilitated cell becomes a new biological and histological unit By the formation of a new structure this cell loses its original function and former appearance, acquiring at least two new characteristics · motility of amœboid type (Sabin, 1923) and the ability to transport antigens in its own cytoplasmic proteins (Godlowski, 1948 *b*) In such circumstances the cell loses the functions of the tissue in which it originated and it migrates from its place of origin by its newly acquired motility.

Relation of Eosinophils to Lymphoid Tissue.

The further fate of such a cell depends on whether it is intercepted by the lymphoid tissue surrounding the portal of entry of the antigen (carried by the migrating eosinophils),

or whether it avoids contact with the lymphoid barrier and penetrates the interior of the organism via the blood (p 14) An example of the first possibility is the migration of *eosinophils* from the cryptal lining of the intestinal mucosa into the interior of the villus, where the lymphocytic barrier of the lamina propria arrests the moving eosinophils (for the mechanism of the arrest see p. 35) An example of the second possibility is the migration of the eosinophilised leucoblastic cell from the bone-marrow into the blood When eosinophils with reproduced antigenic protein in their cytoplasm pass again through the intestinal lymphoid barrier (which is a zone containing specific antibodies in high concentration) they again make contact with the specific antibodies; the resulting union of antigen and antibody causes their arrest. *A zone of high titre of antibodies* surrounds the portal of entry for any antigen (Drinker *et al*, 1941). Such a local concentration of specific antibodies, undiluted by the body fluids, is sufficient to precipitate local antigen-antibody interaction and to immobilise the moving antigen, this local accumulation of antibodies need not necessarily be reflected in the circulating blood

Eosinophils as Source of Antigen.

As soon as eosinophils regain their mobility by re-heparinisation of their gelated cytoplasmic protein (p. 76) they move away into the interior of the organism via lymphatics or blood-vessels Such eosinophils are now deprived of their antigenicity by the antigen-antibody reaction which occurred in the lymphatic barrier, but they still retain the power of re-synthesising the antigenic protein by means of the anabolic phase of their newly formed proteinases Such re-synthesis of the antigenic protein by eosinophils is possible. The renewal of antigenicity in eosinophils is limited by the fact that the eosinophils have moved away from the place where the antigenic protein entered the body, a region where they could receive further supplies of antigen to incorporate into their own cytoplasm. If by chance an eosinophil, having re-synthesised its antigen, should pass a second time through the lymphoid barrier surrounding the portal of entry of its antigen, it would be again arrested and de-antigenised In this way *a permanent cycle is established of de-antigenisation* by the lymphoid tissue

and *re-antigenisation* by the newly formed proteinases of the proteins of eosinophils

Those eosinophils which avoid the barrier of the antigen-antibody reaction, or which have re-synthesised the antigenic protein, represent a potential source of the antigen In normal conditions these cells are either repeatedly de-antigenised in the lymphocytic tissues or, if they make contact with sensitised cells, *they may* precipitate a micro-anaphylactic reaction Eosinophils with *retained antigenicity were removed from the* peritoneum affected by anaphylactic inflammation in experiments on guinea-pigs (Godlowski, 1948 *b*) Such eosinophils, not having made contact with the lymphoid barrier, possessed a strong antigenic power

Eosinophils as Source of Anaphylactic Reaction.

Eosinophils carrying antigenic proteins in their cytoplasm are a potential cause of anaphylactic reactions The antigenic protein, however, being incorporated into the structure of the cell, cannot be liberated until the cell disintegrates This was shown in an experiment in which a suspension of specifically sensitised eosinophils could not produce an anaphylactic reaction in the Schultz-Dale test until they were completely destroyed by repeated freezing and thawing (Godlowski, 1948 *b*) The life-span of eosinophils is not known It is possible that eosinophils, of the same age and carrying the same antigen may disintegrate in the normal ageing process at the same time and in sufficiently high numbers to liberate a toxic dose of antigen, but in normal conditions such possibility is very remote. The minute amount of antigenic protein liberated from a small number of eosinophils represents a sub-toxic dose which can be neutralised by free antibodies in the blood, thus in normal conditions no such micro-anaphylactic reactions take place · *therefore the antigenic capacity of eosinophils under normal conditions, from the point of view of pathogenicity, is small.* If, however, the number of circulating eosinophils is very high, the liberation of antigen may become sufficient to precipitate various degrees of anaphylactic reaction.

Pathogenetic Significance of Eosinophils.

Eosinophils would thus seem to represent a form of cells without specific origin, and with or without antigenic power.

The presence of eosinophils in the blood proves that there is in the organism at least one portal of entry for one or more antigens Since eosinophils are at present regarded as normal elements of the blood there are two alternatives to be considered with regard to their physiopathological state

1 **Anaphylaxis is a physiological phenomenon** it occurs physiologically when an unsplit protein molecule enters the organism by any channel, or originates within the normal organism In such circumstances the pathological manifestations of anaphylaxis begin to appear only when physiological counter-measures normally taken by the organism do not balance the ill-effects caused by the entry of unsplit protein Here the eosinophils, if their number is within normal limits, are normal cells which are unable to proteolyse the invading (physiologically) unsplit protein molecules. This inability of eosinophilised cells results from the *wear and tear* process of their cellular enzymes in normal or in accelerated metabolism. The failing proteinases are thereupon reorganised and so enabled to reproduce the invading protein.

2 **Anaphylaxis is a pathological phenomenon** of universal incidence This latent physiopathology is brought to the light only in its more severe forms In its mildest form anaphylaxis may be regarded as a pathological condition appearing in every organism, and subjecting it to strain which, in turn, accelerates the process of ageing of the intracellular enzymes. The eosinophils in such a concept must be regarded as pathological elements, even if their number is within normal limits By their non-specific origin and noxious character they exhibit a primary imperfection *in the biochemical constitution at the* time of the formation of the organism

GENERAL CONCLUSIONS

THE cells liable to be sensitised are those whose proteolytic enzymes possess insufficient potency to proteolyse the antigenic protein molecules which have invaded their interior. This enzymatic disability may be caused by

1. Primary constitutional abnormality of the enzymatic system, this feature may be organ and species specific. this enzymatic pattern is ruled by the Mendelian law in hereditary transmission
2. A normal ageing process of proteolytic intracellular enzymes.
3. The action of cytoplasmic toxins, directed towards cellular enzymes

To acquire antigenic potency a protein must fulfil the following conditions

1. Its molecules must be smaller than those of the host-cell.
2. Its physico-chemical structure must enable it to stimulate the nucleus of the host-cell for enzymatic adaptation
3. It must escape proteolytic cleavage by the existing enzymes in the invaded organism

The antigenic protein molecules remaining unaltered in the interior of the enzymatically disabled cells strongly stimulate the cytoplasmic proteolytic enzymes to reorganise themselves by the mechanism of enzymatic adaptation The cells which cannot in these circumstances reorganise their proteinases disintegrate completely The cells which are able to reorganise their proteinases become sensitised This intracellular enzymatic transformation constitutes **the primary feature of sensitisation.**

The adaptation or reorganisation of the tryptic enzymes in the sensitised cells is arranged in the following manner. **A specific proteolytic toxic enzyme system** is formed intracellularly by the mechanism of *coupled reaction*, this system consists of the nuclear *organising factor* and molecules of the antigenic protein. This enzymatic system cannot start proteolysis until and unless it is augmented by another molecule of the same antigenic protein; this augmentation occurs in the second (shock) invasion of antigen

In the **initial stages of anaphylactic shock** the toxic hydrolysis yields various proteoses intracellularly which, by intrinsic action, disrupt the intracellular lipo-protein molecules thereby exposing structural proteins of the cytoplasm to tryptic hydrolysis In the later stages of *anaphylactic shock the* proteoses, by extrinsic action, inactivate lipoids in the lipoprotein conjugation in the cells not primarily affected by the anaphylactic reaction. This action starts chaotic proteolysis of toxic and non-toxic types and liberates toxic catabolites which are responsible for the manifestations of the anaphylactic reaction

Thus the initial and primary stages (in the intrinsic phase) of the anaphylactic reaction take place in the sensitised tissues; *in the extrinsic phase of the anaphylactic reaction the whole* organism is involved in the " proteolytic storm "

Antigen-antibody union, *per se* a non-noxious reaction, is a defensive measure which takes place mainly in the lymphoid tissue and in the body fluids , it has the task, in the first place, of preventing the access of antigen to cells with an inadequate proteolytic system, *i e* , it prevents sensitisation In the second place, it prevents the access of antigen to sensitised cells and so prevents *anaphylactic shock*

Proteolytic antibodies may be regarded as those proteinases which appear in the cells as a result of antigenic stimulation and which can proteolyse only the antigenic molecules to **the level of non-toxic catabolites.**

Toxic proteolytic enzymes are those proteinases which appear in the cells and next in the body fluids as a result of antigenic stimulation, and which can hydrolyse only the antigenic protein *(specific) to the level of toxic catabolites.*

Various **toxic intermediate catabolites** of protein hydrolysis are set free during the uncontrolled anaphylactic proteolysis and, liberated into the blood, they exert their extrinsic toxic activities. *The chemical* stress of this action, in turn, mobilises *the* pituitary-adrenal response which releases a great variety of corticosteroids Released C^{11}-oxygenated corticosteroids maintain the proteolysis which has been initiated by proteoses liberated *intracellularly in the early* stages of the anaphylactic reaction The manifestations derived from this *endocrine involvement* are superimposed upon the symptoms produced originally by the anaphylactic reaction

The quality of the released toxic protein catabolites *determines* the character of the anaphylactic reaction; the type

of catabolites released depends primarily on the chemical composition of the hydrolysed proteins The main toxins released into the body fluids during the anaphylactic reaction are heparin, histamine, and various proteoses

Heparin released from protein salts in the early stages of the anaphylactic reaction causes incoagulability of the blood, in the recovery stage of the anaphylactic reaction a tendency towards an increased coagulability supervenes as a result of the rapid withdrawal of heparin from the blood The de-heparinisation of the blood proteins is caused by re-heparinisation of the proteins of the tissues involved in the anaphylactic reaction This increased coagulability favours the formation of intracapillary clotting which is responsible for *the ischæmic necrosis* so characteristic of the later stages of anaphylactic inflammation

Histamine released during the anaphylactic reaction is responsible for most of the dramatic manifestations of anaphylactic reactions by its intrinsic and extrinsic actions.

Catabolites of the higher level of protein hydrolysis (proteoses) released during the anaphylactic reaction (leucotaxin, leucocytosis promoting factor, leucopenic factors, pyrexin, necrosin, etc.) are responsible for various local and constitutional manifestations.

Symptoms identical with those of the anaphylactic reaction can be produced by application of agents capable of initiating chaotic and uncontrolled intracellular proteolysis. These agents are.

1. Physical · intravenous injection of fat solvents.
2. Physico-chemical intravenous injection of colloidal solutions, *e g*, protein, peptone, sulphur, etc, or non-colloidal suspensions, *e g*, kaolin.
3. Chemical : intravenous injections of oxidising agents such as hydrogen peroxide, iodides

Intracellular and extracellular proteolysis can be initiated *in vivo* and *in vitro* through the disruption of the lipo-protein molecules, lipoids in lipo-protein molecules protect the protein components against an interaction with the protein of proteolytic enzymes These lipoids may be rendered inactive in the following manner by the same agents as those which precipitate anaphylactic-like syndromes:

1 By removing the lipoid barrier separating the substrate

proteins from proteins of enzymes by fat solvents such as chloroform, acetone, etc. (physical action).

2. By altering the dispersion phase of the lipoids in the lipo-protein molecules by surface-acting substances such as colloidal solutions or non-colloidal suspensions (physico-chemical action).

3 By breaking the double bonds in the unsaturated fatty acids of the lipoids, e g , through oxidation by hydrogen peroxide, halogenation by iodides, etc (chemical action).

The stress of the anaphylactic reaction releases the pituitary-adrenal *response* · liberated corticosteroids interfere with various phases of peripheral metabolism , those steroids which are *involved* in protein metabolism initiate proteolysis by the disruption of the lipo-protein conjugation in those tissues which are rich in proteolytic enzymes.

Lipo-protein conjugation has the task of protecting the substrate proteins against undue hydrolysis carried out by the proteolytic enzymes normally present in all organs Any action which disrupts the lipo-protein molecules is apt to release proteolysis of the organism's own proteins Rapid disruption of the lipo-protein conjugation leads to partial hydrolysis which leaves behind highly toxic protein catabolites.

C^{11}-oxygenated corticosteroids disrupt the lipo-protein conjugation by saturation of the double bonds in the fatty acids *of the lipoids*: this action is closely co-ordinated with the *tryptic capacity* of the proteolytic enzymes available this proteolysis leaves no toxic degradates of *protein.*

***The beneficial effects* of the C^{11}-oxygenated corticosteroids in allergic conditions** are produced by *elimination of the* secondary histo-chemical changes in the affected tissue, *these* changes take place in the third stage of *anaphylactic shock,* and the *steroids remove them* by *initiating the proteolysis* of the accumulated inflammatory exudates *this proteolysis,* however, leaves the *primary mechanism of the first* and second stages of the anaphylactic shock unaffected.

The process of *formation of eosinophils* or eosinophilisation takes place in those cells which do not possess *sufficiently* active proteinases. *These cells, invaded by* unsplit protein molecules, reorganise their intracellular enzyme *systems* under the stimulating action of foreign *protein molecules* *The newly* reorganised proteinases in their *catabolic phase* reconstitute

the invading foreign protein and incorporate it into the structural proteins of cytoplasm Thus eosinophils may become a source of antigen

Eosinophils are cells which are formed physiologically by the same mechanism in the bone-marrow, in the mucosa of the alimentary canal, or in any other non-hæmatopoietic tissue in which the conditions for eosinophilisation may exist. Those formed in non-hæmatopoietic tissues may be designated as *pseudo-eosinophils* because, according to embryological classification, they do not belong to the true blood elements.

Antigenic protein when absorbed in the intestine may cause not only eosinophilisation in the cryptal epithelium but also may stimulate the intestinal lymphoid tissue barrier to produce specific antibodies These two effects are produced by different parts of the same antigenic protein Thus eosinophils containing antigen in their cytoplasm, while moving through the lymphocytic barrier, meet a high titre of specific antibodies. As the result of the antigen-antibody reaction (antigen of eosinophils and antibodies of lymphoid tissue) *eosinophils are temporarily arrested* there and *temporarily deprived of their antigenicity* The arrested tissue eosinophils can be mobilised when the arresting mechanism of the antigen-antibody reaction becomes exhausted Eosinophils deprived of their antigenic potency are thus mobilised and shifted into the circulation. Here, however, the antigenicity of eosinophils may be restored by the anabolic action of their newly reorganised proteinases.

During the **eosinopenia induced by hormones** circulating eosinophils are destroyed in the blood by the lytic action of C^{11}-oxygenated corticosteroids which accelerate their intra cellular proteolysis By contrast the tissue eosinophils, because of their altered physico-chemical state, are not susceptible to the eosinolytic action of corticosteroids. All other white blood cells are affected in a similar way by the same action of corticosteroids, but the coexisting mobilisation of polymorphonuclear leucocytes masks their destruction.

Eosinophils are to be regarded as carriers of various antigens. If large numbers of these cells are destroyed, their antigenic protein may be liberated in sufficient amounts to precipitate an anaphylactic reaction But a destruction of eosinophils sufficiently massive to cause an anaphylactic reaction must be a rare occurrence

Eosinophils are examples of isolated cells which [illegible]

a new function—*that of synthesising foreign protein;* this activity of eosinophils is maintained *in* physiological as well as in pathological *conditions* The basic difference between these two conditions lies *(so far as the function* of eosinophils is concerned) *in* the type of the synthesised *protein* A fuller study of eosinophilisation *(a* phenomenon of minor significance *in itself) may shed light* on the enzymatic synthesis of protein in general and neoplastic protein *in* particular.

The presence of anaphylactic phenomena, whether histo-*chemical or* constitutional, can be traced in all living organisms. Eosinophils may be found in the very young embryo, *a finding* which should be looked upon as evidence of anaphylaxis. *The difference* between the physiological and pathological anaphylactic *response* lies in the *type of antigen* which *induces* this response and in the *degree* of the response *The* presence of anaphylactic symptoms in normal individuals may be regarded, therefore, as *the physiological response of a normal* organism to stimuli inflicted upon *it* by the environment. This **physiological anaphylaxis** may be *aggravated* if *the immunological counter-measures (in anaphylactic reaction)* taken by the organism against these *stimuli* are inadequate

Thus eosinophilisation is regarded as a result of the *inadequacy of the intracellular proteolysis which also* forms the basis of anaphylactic reaction; hence eosinophils may be looked upon as a histo-chemical evidence of anaphylactic response *Anaphylactic response, in turn, is an expression* of either normal or pathological exhaustion *of* the proteolytic capacity of the enzymatic system So long as *the aged enzymes* are replaced by *normal adaptive enzymes, the* whole process may be regarded as beneficial because it provides the cell with a new potent enzyme · it "rejuvenates" the cellular enzymes. When the regenerated *adaptive enzymes* acquire *a toxic character, this* process involves *the* cell in a vicious circle which constitutes the basis of sensitisation The pathology which *results from* this involvement is shaped by *(1) the character of the antigen which has induced* sensitisation, (2) the breakdown products which are liberated during the toxic proteolysis, and (3) the predisposing factors of the involved *organ (e g.,* anatomical structure, *superadded* infection, etc.).

In other words, clinical allergy is an aggravated *response* of physiological anaphylaxis, which *is conditioned* by locally coexisting predisposing factors

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